Individual variation in onset of behavioural traits in the domestic cat (*Felis catus*) as assessed by owner/carer survey.

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Abstract

The prenatal maternal environment is known to play a role in lifetime development of a large number of species but has received relatively little attention in the domestic cat (*Felis catus*). We surveyed anatomical and behavioural onsets in 251 home reared kittens from 66 litters from pedigree (n=28) and non-pedigree queens (n=38) during the first 8 weeks of life using owner/carer records. Multi-factorial analysis was used to assess associations between a range of factors including: breed, age and temperament of the queen (Queen's Friendliness Rating or QFR); size and sex ratio of litters; and individual birth weight on a number of developmental milestones including: onset of eye opening; tooth eruption; movement from nest; play; solid feeding; and use of litter tray.

Pedigree Asian breeds produced larger litters with lower average birth weight than pedigree Western breeds or non-pedigree queens. Kittens from larger litters (5 or more kittens) opened their eyes earlier than smaller litters, however, they had a generally delayed onset of subsequent developmental milestones with the exception of use of the litter tray. Kittens from older queens (over 2 years of age) showed earlier onsets of eye opening, but later tooth eruption, play and movement from nest than kittens derived from younger queens.

Queens rated as being unfriendly to humans produced litters with a female bias. Litters from friendly queens showed earlier tooth eruption, solid feeding and litter tray use but delayed object play compared to litters from less friendly queens. These results support previous work on the influence of breed and litter size on kitten development, but also indicate age and temperament of the queen may influence developmental milestones. The picture is however complex, with

advanced early milestones (such as eye opening) often associated with delayed later milestones such as locomotion and play.

Key words

Kitten development milestones, cat breed, queen temperament

Contents

Abbreviations used in Thesis

ACTH: Adrenocorticotropic hormone

AOB: Accessory olfactory bulb

CNS: Central Nervous System

CRH: Corticotrophin-releasing hormone

GR: Glucocorticoid receptors

HPA: Hypothalmic-Pituitary-Adrenal axis

MR: Mineralocorticoid receptor

mRNA: Messenger RNA

NFC: Norwegian Forest cat

OSA: Oriental/Siamese/Abyssinian

PNS – Prenatal Stress

PVN - Paraventricular nucleus

QFR. Queen's Friendliness Rating

Chapter 1. Introduction

1.1. Environmental influences on ontogeny in cats

Ontogeny or the development of anatomical and behavioural features is under considerable genetic control, but these features and their onset are not developmentally fixed. Environmental factors also have a profound effect on development, from the internal environment surrounding the embryo and foetus, to after birth where external variables such as daylight (e.g. Braastad & Heggelund 1984), climate (Broom & Johnson 1993), food availability (Bradshaw 1993) and socialisation within sensitive periods (McCune 1995) influence individual phenotype (Braastad & Heggelund 1984; Bateson 2000 for review in domestic cats). There has been increasing interest in how the interplay between genetic and environmental factors affect individual variation in behavioural traits in a number of species, including an understanding of the relationships between genotype and environment including prenatal and post-natal epigenetic effects (non-genetic influences on gene expression) and their consequences for individual differences in behavioural responses, lifetime fitness and quality of life.

Much of this research has focused on human development. Kikusui & Mori (2009) suggest that early life experience has a marked affect on the developing structure of the central nervous system (CNS), thus influencing behavioural outputs; for example, abused children develop with a decreased hippocampus and corpus callosum, but enlarged cerebral ventricles (Bremner *et al*, 2003). Many of these advances are relatively novel even in our understanding of human development, and certain elements, including epigenetic influences have only recently been applied

within the field of animal welfare and behaviour (e.g. Rutherford et al, 2009; Foyer et al, 2016).

Animal models have often been used to assess the impact of changing early life environmental factors on development, in particular the consequences of adverse conditions on subsequent behavioural development. These can illustrate the scope of individual variation in phenotypic traits and the range of factors that contribute to this variation. For example, Braastad & Heggelund (1984) in their study of behavioural development in the domestic cat highlighted how the developmental period of eye opening can be influenced by genetic factors such as paternal genotype and sex of the kitten; and by environmental factors including exposure to light, the age of the mother and the onset of eye-opening in the kittens' littermates. They found that kittens showed considerable variation in onset of eye opening, which began between 1 and 13 days after birth (Braastad & Heggelund 1984). When data was subjected to multiple-regression analysis, it revealed that four factors; paternity, illumination, sex of kitten and mother's age contributed to individual variation in the onset of eye opening. Those reared within a darkened environment began to open their eyes earlier and also took less time for both eyes to be fully open. In addition, kittens whose mothers were classed as young had an earlier initiation of eye opening. There was also a sex difference in onset of eye opening with female kittens opening their eyes earlier than male kittens. Due to the nature of their methodology, maternal influences, both behavioural and genetic were not accounted for and may also serve as a significant contributor towards differences in initiation and completion of eye opening. Other researchers note additional factors that may contribute to eye opening in kittens; Beaver (2003), for example, describes how eye

opening in domestic cats can be accelerated by early handling, which had also been reported by Hart (1976).

Genetic influences on individual variation in behaviour of cats have been suggested in studies which have compared breeds; for example, increased incidence of pica (ingestion of non-food items such as wool) is reported in Oriental breeds (Bradshaw et al 1997). Few studies have investigated the development of these breed specific traits (Bateson 2000) under controlled conditions. An exception is the work of Marchei et al (2001) who compared the behavioural, anatomical and physiological development of Oriental/Siamese/Abyssinian (OSA) kittens with Norwegian Forest (NF) kittens. The mean age of eye opening was significantly earlier in OSA cats compared to NF kittens, whereas NF kittens developed thermoregulatory abilities at an earlier age than OSA kittens. For behavioural testing, Marchei et al (2001) used open field tests with and without potentially threatening objects. They found that there were significant differences between the two breed categories in behaviour during these tests. NF kittens had delayed limbic development, poorer memory and slower habituation to novelty compared to OSA kittens when observed in open field testing. NF kittens were described as exhibiting a more active coping style when presented with potentially threatening objects as they were more inclined to seek escape routes than OSA kittens. The OSA kittens in contrast were described as adopting a more passive coping style as they displayed lower exploratory behavioural responses and fewer vocalisations in response to a novel object during open field tests (OFT). However, such behavioural testing and results should be viewed with some caution as validation of the potentially fear-eliciting item utilised was exempt and may not be representative of aversive stimuli found in the real world setting. Furthermore, kittens were subjected to novelty solely

by being individually placed in the OFT (omitting the stimuli) at an age before weaning which may also contribute to the findings in the study.

A major influence on prenatal and post-natal development is the maternal environment, including the prenatal environment of the developing foetus, post-natal care and the consequences of maternal deprivation during these periods. Factors such as placental insufficiency, and challenges due to disease and/or nutritional deficiency can play significant roles during development (Matthews, 2002). This can be illustrated by the effect of poor nutrition in the mother, which can affect the size and survival of offspring, and their behavioural development. For example, kittens suckling from a food deprived mother (>80% of nutritional requirements) can show developmental deficits in the CNS including an overall lowering in brain size and delays in maturation in key regions specifically the cerebrum, cerebellum and brain stem (Bradshaw, 1992). An example of a behavioural response to a specific nutritional deficit can be seen where lack of protein in the diet leads to motor defects in both males and female kittens, and an increase in aggressive tendencies in male kittens (Bradshaw, 1992). More subtle behavioural effects (e.g. offspring's preference for palatable foods), from dietary differences between mothers are also beginning to be revealed in a range of species including humans and rodent models, arising from epigenetic changes relating to methylation and histone acetylation of the genome (McGowan, et al 2008; Vucetic, et al 2010).

As well as directly affecting developmental milestones in the growing kitten, nutritional deficiencies and differences can have wide-ranging and long-lasting effects through epigenetic changes, where early environmental experiences alter

expression of genetic traits. It has been suggested that metabolic programming in offspring whose mothers were nutrient deprived during pregnancy can lead to physiological adaptations that help the offspring cope with a poor nutritional environment during their lifetimes (Campion et al, 2009). For example, altered pancreatic cells secrete insulin in response to ingestion of very small quantities of food, to ensure as much nutrient can be stored by the body possible (Parlee & MacDougald, 2014). Major issues can arise if the offspring lives in an environment where food is more plentiful; in such a situation the body may not reprogram itself leading to increased risks of suffering illnesses such as obesity, hypertension and diabetes (Parlee & MacDougald, 2014).

These examples illustrate the potential influence of nutrition on the domestic cat queen during gestation and lactation via both the anatomical and behavioural development of her kittens. The experimental chapter of this thesis will explore other factors that may affect kitten development including the breed and age of the queen, and the number and sex ratio of the kittens. The sample population being made up of pedigree breeding and stray queens also provided an opportunity to explore the potential impact of queen's temperament with respect to human interaction by developing a Queen's Friendliness Rating (QFR). Before presenting this study, it would however be useful to review what is known about the impact of factors that may impact on kittens' behavioural development, in particular prenatal maternal stress and post-natal care. The review will draw from research across a range of species, but with reference to the domestic cat where relevant.

1.2. Prenatal stress and foetal environment

Prenatal stress (PNS) refers to the impact of environmental stressors in utero, mediated through the developing individual's maternal environment. Scenarios

where the mother and consequently her offspring in utero are exposed to high levels of stress include environmental changes not normal to that species, noxious events or human intervention, for example, the capture and housing of pregnant under-socialised domesticated species, such as the cat. Prenatal stress (PNS) has considerable potential to influence ontology, including the behavioural development of offspring, such as the modification of behaviour, cognition, memory, learning and emotionality (Del Cerro *et al*, 2010), in addition to physiological changes such as glucose regulation, cardiovascular and immune systems (Matthews, 2002). Kapoor *et al* (2006) demonstrated that challenges within the maternal environment such as predation activate the maternal stress response. Hougaard *et al* (2005) found that circulatory gluco-cortiocoids were able to pass from rat dams to the foetus via the placenta, and were partly involved with phenotypic alterations as a result of PNS.

The impact on the growing foetus of environmental stressors can be wide ranging in their nature and severity. For example, severe maternal stress, trauma and/or poor nutrition can result in re-absorption of foetuses, mis-carriage or delayed implantation, with exact consequences varying between species and the nature and form of challenges (Chen, 2011; Qu, 2017). There has, however, also been a growing body of research that suggests that milder stressors, can have positive impact on the developing individual, as a potential adaptation to environmental challenges; one route that has received particular attention is the impact of the maternal stress response on developing foetus, causing adaptation and modification of its hypothalamic-pituitary-adrenal (HPA) activity. For example, Roussel *et al* (2004) proposed that ewes' maternal behaviour, such as habituation to mild stressors, may act as an adaptive mechanism to counteract the effects stress have on the growing foetus by decreasing circulatory glucocorticoids. They also suggested

that elevated maternal glucocorticoids as a result of mild stressors increase maternal food consumption and placenta nutrient transporter capacity which may act as physiological adaptation to support the foetus (Roussel *et al*, 2004). This may account for increased birth weights noted within some studies, where mothers are exposed to mild stressors, such as that by Muller & Bale (2006), discussed later in this chapter. As an adaptive mechanism mild PNS can increase stress management within offspring. This has been demonstrated in rats, where low levels of corticosterone added to the drinking water of pregnant dams resulted in offspring with an ability to better handle stress as adults. This was associated with reduced fearfulness and improved learning capabilities which has been related to increased glucocorticoid receptors (GR) within their hippocampus (see review Catalani *et al*, 2011). It would however be spurious to suggest that the impact of prenatal environmental stressors is always adaptive and where maternal stress exposure does not mimic that of its offspring or where there is chronic stress as a result of major repeated traumatic experiences, PNS has the potential to lead to physiological dysfunction and reduced welfare of progeny in adulthood, if the pre-programmed phenotype of the offspring in utero does not match the environment in which it will live (Boersma & Tamashiro for overview in rodents, 2015).

PNS has been linked to the increased release of corticosteroids during gestation. Circulatory glucocorticosteroids are able to pass from the mother to the foetus; therefore, higher levels of circulating maternal gluco-corticosteroids increase foetal exposure (Otten *et al*, 2010; Catalani *et al*, 2011). Foetal exposure is broadly dependent on the following factors: a) the timing and duration of exposure during gestation; b) species specific neuro-endocrine development of the CNS and brain regions; and c) individual variation in the mother's response to stressors or her

temperament. The mother's ability to deal with a stressor will ultimately be dependent upon her own genotype and previous experience (Roussel *et al*, 2004; Kapoor *et al*, 2006) and the nature of the stressors. Novel stressors, or those that are unpredictable, uncontrollable, or that inflict extreme or inescapable fear or pain may produce a greater stress response when compared to stressors that are more easily habituated to, such as gentle handling or where the animal has a sense of control over its environment (Broom and Johnson, 1993; Charil *et al*, 2010). For example, Fride & Weinstock (1984) demonstrated that rat pups derived from mothers who were exposed to thrice-weekly randomly timed stress events showed a delay in behavioural development compared to offspring derived from rat dams that were exposed to the same stress events regularly throughout pregnancy. In non-experimental animals in the field, maternal stressors may include unpredictable disruption to feeding routines, resource deprivation including barren living conditions, maternal health status, variation in air temperature and prolonged/chronic noxious experiences (Broom and Johnson, 1993).

1.2.1 Timing of Prenatal Stress

Timing of exposure to PNS during gestation has been documented to influence behavioural and physiological development in offspring. For example, pinpointing timing of PNS during gestation and its effects has been analysed within a number of species, in particular the guinea pig (*Cavia porcellus*) as an example of precocial species (Lingas & Matthews, 2001), and the domestic rat (*Rattus norvegicus)* as an example of altricial species (Fride & Weinstock, 1984; Estanislau & Morato, 2006; Xiaobai *et al*, 2014) Though some literature pertaining to PNS timings has also been

documented in goats (*Capra aegagrus hircus)* (Roussel *et al*, 2005), pigs (Kranendock *et al*, 2005; Otten *et al*, 2010) and primate species such as rhesus macaques (*Macaca mulatta*) (Clark *et al*, 1996).

An example of the effects of varying the timing of exposure to PNS is provided by Wellburg *et al* (2001), albeit using a synthetic manipulation of hormonal state rather than using environmental stressors. This study assessed the effects of PNS at different stages during gestation by the use of the synthetic glucocorticoid, dexamethasone. They administered dexamethasone to rats either throughout pregnancy or during the last trimester of gestation. Both treatment regimes produced offspring with increased baseline corticotrophin-releasing hormone (CRH) mRNA in the central nucleus of the amygdale. The offspring from both treatment groups also displayed reduced exploratory behaviour in open field tests compared to controls. The amygdala, located in the temporal lobe of the brain, is associated with emotional events, specifically fear and frustration. The central nucleus of the amygdala, which receives afferents from the brain's sensory systems via the basolateral nuclei of the amygdala specifically triggers the sympathetic nervous system via the hypothalamus which is involved in initiation and moderation of the response to environmental stressors. Wellburg *et al* (2001) suggested that increased levels of CRH within the central nucleus of the amygdala account for anxiogenic behaviours such as the reduced exploration witnessed in open field tests.

Wellburg and colleagues (2001) also found that offspring of rat dams administered with dexamethasone only during the third trimester of gestation exhibited reduced coping and learning capabilities in a forced swim test and elevated anxiety related behaviours such as avoidance of exposed arms in an elevated plus

maze. These offspring were found to have significantly lowered glucocorticoid (GR) and mineralocorticoid receptor (MR) mRNA within the hippocampus, the body that is associated with memory formation and consolidation during learning. These rat pups also exhibited increased CRH mRNA but showed unaltered GR mRNA within the hypothalamic paraventricular nucleus (PVN). This structure receives inputs from the hippocampus and both the hippocampus and the PVN are involved with the negative feedback loop associated with the control of release of glucocorticoids. These changes would lead to inhibition of the negative feedback loop of the HPA axis, leading to prolonged or elevated physiological and behavioural responses to environmental stressors. In contrast, individuals born to rat dams administered with dexamethasone throughout the gestation period showed no changes in prevalence of hippocampal MR or GR, but an increase in corticosterone receptors in the basolateral nucleus of the amygdala.

Wellburg *et al (*2001) therefore suggested that reduced GR and MR within the hippocampus of offspring, induced by a rise in exposure of glucocorticoids within the third trimester of gestation, played a role in impaired learning and memory functions in the growing rat pup. They also inferred that the expression of CRH mRNA in the central nucleus of the amygdala is facilitated by raised glucocorticoids, perhaps due to the increased number of receptors in the basolateral area producing anxiogenic effects. However, it should be noted that unlike endogenous glucocorticoids, synthetic glucocorticoids are not converted to an inactive product by 11-beta hydroxysteroid dehyrogenase (Kapoor et al, 2008). Emerging evidence suggests that whilst synthetic glucocorticoids can be removed via p-glycoprotein transportation, p-glycoprotein activity wanes during late gestation and there may therefore have been increased foetal exposure to synthetic

glucocorticoids within this period (Kapoor et al, 2008) which may account for the behavioural changes witnessed within this study, specifically when treatments were administered solely within the third trimester of pregnancy.

1.2.2. Prenatal Stress: Effects on precocial and altricial species

The effects of PNS are thought to be species specific, although species can be categorised in a number of ways to draw cross-species conclusions. One way of categorising species which may be relevant to their response to prenatal and post-natal stressors is whether they can be classified as precocial or altricial. Precocial species are born relatively mature, often capable of independent feeding and movement within hours of birth or hatching, e.g. ducks, guinea pigs & grazing animals. In contrast altricial species are born relatively immature and are heavily reliant on maternal care during early infancy, e.g. mice, rats, cats & humans (Kapoor *et al*, 2006). In general, neurological functions within precocial species are more fully developed by the end of gestation, whereas there is significant further development of altricial species during late gestation and into the post-natal period due to differences in brain, motor pattern and neuroendocrine maturation (Darlington et al, 1999; for review see Kapoor *et al*, 2006). Whilst cats are a good example of an altruitial species, being born "blind and helpless" and heavily dependent on maternal care (or requiring intense human intervention if this is absent), as there has been little research into potential impact of prenatal stress in cats, this section shall describe in detail some relevant studies of more intensively studied altruitial (primarily studies in rats) and precocial (mainly guinea pigs) species to illustrate the potential differential impact of PNS across species.

In both precocial and altricial species, it is the developmental period between the first appearance of MR and GR within their CNS and full maturation of the HPA axis when susceptibility to PNS is most apparent (Weinstock, 2008). As a significant proportion of the neuroendocrine development of altricial species continues into the post-natal period, maternal care after birth in addition to PNS may therefore influence stress susceptibility in adulthood to a greater extent than precocial species (Weinstock, 2008). In contrast, the effects of PNS on precocial species are found to be more pronounced when exposure to stressors occurs during early to mid gestation. For example, adult male guinea pigs born to mothers that have experienced stressors during gestation exhibited alterations in their ability to handle stress. Those exposed to PNS at an early gestation stage exhibited a greater susceptibility to stress and weighed significantly less as adults than those exposed in late gestation (Kapoor & Matthews, 2005). This finding is consistent with Schopper *et al* (2012) who demonstrated that guinea pig dams exposed to PNS during early to mid gestation produced offspring with higher basal glucocorticoid levels than control animals post adolescence.

The timing of PNS may affect male and female offspring differently. For example, when comparing differences to stress susceptibility between male and female guinea pigs, Emack et al (2008) found that chronic maternal stress exposure in mid to late gestation had greater impact on the development of male offspring than females. To do this they exposed dams to one of four stressors every second day in a randomized order, from gestation day 32 to day 66, then again from post-natal day 1 to weaning. They assessed birth weight, growth, cortisol and behavioural parameters and concluded that both females and males born to PNS dams had

elevated salivary cortisol levels at post-natal day 25. However, the morphological and behavioural consequences of maternal stress were only displayed in male offspring as they exhibited lowered growth rates, reduced head circumference and reduced open field ambulatory activity compared to control males. In contrast female offspring born to dams exposed to PNS showed none of these developmental impairments. However, it should be noted that this study did not compare the effect of chronic stress between early and late gestation within this species.

In altricial species there has been a focus of research on the effects of PNS in late gestation, which maps onto the acceleration of neuro-endocrine development known to occur within this period of pregnancy, for example using the rat as a model, it has been documented that PNS during the later stages of gestation reduced the density of GR and MR within the hippocampus, leading to increased circulatory corticosteroids in response to stressors (Barbazanges *et al* 1996) and predisposing the rat pups to increased stress vulnerability (Green *et al* 2011). However, Fride & Weinstock (1984) demonstrated that changes in the timing of behavioural development and eye opening were dependent on when PNS was induced. Rat dams who were subjected to random or unpredictable stressors, in the form of changes to light regime throughout gestation period produced offspring with delayed behavioural and physiological development after birth. In contrast, offspring derived from dams subjected to this unpredictable stressor in the last week of gestation showed an acceleration in such development, though no change in time of eye opening. They also studied rat dams who received regular (or predictable) stressors throughout gestation where it was found that there was no change in developmental milestones, however their pups weighed more at birth although litter size was

considerably reduced. The increased speed at which development occurred, recorded by Fride & Weinstock (1984), as a consequence of PNS exposure in late gestation, coincided with the period of pregnancy when there is a natural surge in circulatory glucocorticosteroids in the developing foetus, necessary for organ system development within the prepartum period. Although both altricial and precocial species exhibit an increase in foetal glucocorticosteroids in the later stages of gestation, precocial species tend to show greater elevations in preparation for independent living post-partum (Kapoor, 2006). Nevertheless, altricial species such as the rat are also susceptible to enhanced development as mediated by additional glucocorticosteroids due to PNS during late gestation as evidenced by Fride & Weinstock's (1984) study.

Kapoor et al (2006, 2008) argued, that to moderate undesirable consequences of high levels of endogenous corticosteroids on early development it is thought that an underlying adaptive mechanism, termed the stress hypo-responsive period, inhibits detrimental elevations of endogenous glucocorticosteroids in the developing foetus/neonate (Kapoor et al 2006). In this, endogenous glucocorticosteroids are converted by 11-beta hydroxysteroid dehyrogenase to an inactive ketone product, moderating glucocorticosteroid levels post-partum or decreasing the placenta transfer of maternal glucocorticosteroids to the foetus (Kapoor *et al*, 2008). This ensures that a lower level of corticosteroids is maintained for normal physiological development (Bakker *et al*, 2001). Although predicted to have value across a range of species, little is known about when this period occurs within many species of mammal including the domestic cat. Within the rat (an altricial species) this period is between post-natal days three and fourteen, suggesting that other physiological

mechanisms are active as placental transfer will no longer mediate a regulatory action (Bakker *et al*, 2001). Kapoor et al (2006, 2008) argued that for those species where the stress hypo-responsive period is located before birth, PNS should in theory have a greater effect on offspring. Within humans this period is thought to occur during the third trimester of pregnancy, and in precocial species during both the pre and perinatal periods (Kapoor et al, 2006).

1.3. Effects of Prenatal Stress

The effects of PNS on offspring has only been researched in a number of species, including guinea pigs, rats, Rhesus macaques, pigs, and blue-fox cubs (Braastad, 1998; Takahashi 1998; Kapoor et al, 2006). The influence of PNS on the behaviour of offspring of farm and laboratory animals has been advanced by Braastad (1998), where historical variation within research methodologies was highlighted, though more recent studies have implemented specific paradigms to narrow these differences (e.g. Gotz, *et al*, 2008).

PNS has been associated with impaired memory (Lemaire, *et al*, 2000) as well as altered circadian functions responsible for the regulation of the HPA axis in a number of species. Examples include: corticosterone secretion in adult rat offspring (Koehl et al, 1999); the ability to regulate HPA activity in adult offspring resulting in the exhibition of anxiety related behaviours in various species (Barbazanges, *et al*, 1996, Henry, *et al*, 2004; Green, *et al*, 2011); and effects on the immune response, documented within domesticated pigs (Tuchscherer, *et al*, 2002; Couret, *et al*, 2009). It has also been suggested that prenatal stress may have a direct influence on the number of males and females born within a litter (Navara & Nelson, 2009).

1.3.1. Sex Ratio in Litters

Navara & Nelson (2009) describe the manipulation of sex ratios as a means to maximise fitness potential as a result of the influence of environmental variables, which include food abundance, seasonal and climatic influences and social dynamics. This theory originated from Trivers and Willard (1973) who predicted that mothers in good condition will invest in the sex with the highest fitness. For example, in polygamous species, where males mate with multiple females, mothers who are food deprived produce a higher female to male ratio of offspring as poor quality males would be unlikely to maximise their breeding potential, whereas poor quality females still had a chance of contributing to subsequent generations to their potential. Conversely in an environment where food is abundant and the population of the species is thriving, investment in male offspring will have selective advantage. In species that are polyandrous (species where females mate with multiple males) this is often reversed. This implies both post-copulatory sexual selection occurs by females as well as post-natal differential investment.

More recent research has challenged this hypothesis; a meta-analysis conducted by Cameron (2004) found that the majority of studies did not provide adequate evidence to support the hypothesis due to experimental design and differences in data parameters. She did however conclude that when measures of maternal body condition were taken at conception (rather that at other points during the reproductive cycle), the majority of studies analysed (74%) supported the Trivers-Willard hypothesis.

Cameron (2004) also commented on the role of glucose within this paradigm, specifically that different levels of circulatory glucose may affect sex ratio. Cameron (2004) referred to research conducted by Rosenfeld et al (2003) who found that

more male mice pups were born to mothers that were fed a high fat diet around conception, whereas when a low-fat diet was fed, an increase of female mice pups was witnessed. Similarly, Helle *et al*, (2008) incorporated the role of testosterone within their investigations of sex bias, where they discovered that female field voles that had elevated serum glucose and testosterone levels produced litters with a male bias and that voles that produced larger litters tended to have a greater proportion of males within the litter. However, they reported that neither maternal body condition nor maternal corticosterone levels had an effect on gender bias. This contrasts with previous studies by Pratt & Lisk, (1989) and Pike & Petrie, (2005; 2006) that reported that increased maternal corticosterone levels affect litter gender bias in avian species. Unfortunately, such detailed research on the effects of maternal corticosteroid levels on litter sex bias in mammalian species is extremely limited and this would be an area to investigate further. In contrast, behavioural differences as a result of varying litter sex ratios have been widely reported. For example, when behaviourally assessed, single males within litters of female mice display greater inter-male aggressive tendencies later in life when compared to males reared in a male only litter (Namikas & Wehmer, 1978).

1.3.2. Birth weight and litter size

Several studies have suggested that PNS has a direct effect on birth weight, (e.g. Butkevich, *et al*, 2009, Welburg, et al, 2001) and post weaning weights of rat pups (Kapoor & Matthews, 2005). Furthermore, timing of PNS during gestation influences weight; Rhesus monkey infants subjected to early gestation PNS were found to weigh less than individuals exposed to mid-late gestational PNS (Schneider, et al, 1999). Conversely, there are also reports of no difference in birth

weight between PNS offspring and controls (Tuchscherer, et al, 2002) nor overall body weight (Bhatnagar, et al, 2005). Increases in rat pup weight have also been reported at time of birth (Roussel, *et al*, 2004; Mueller & Bale, 2006), as well as during subsequent development, both at weaning and at 30 days of age (Estanislau & Morato, 2006), suggesting that the type of stressor and subsequent stress response dependent on individual reactivity could play an important role in determining outcomes in offspring.

Muller & Bale (2006) suggested that reduced birthweight associated with maternal stress in late gestation (repeated predictable stress) was due to stress directly reducing maternal food intake. As a consequence, they employed a variable chronic stress model to induce PNS but without directly affecting maternal feeding behaviour or inducing pain. They found that birth weights of wild-type PNS mice were significantly higher than control wild-type mice, though weight of PNS wild-type mice normalised in adulthood (Muller & Bale, 2006). In another study, where mild repeated stress was inflicted on sheep ewes during the last 5 weeks of gestation, offspring also had increased birth weights compared to controls (Roussel *et al*, 2004).

Litter size may also be affected by prenatal environment in a number of contrasting ways. A mother can have a larger number of individuals when she has the resources to support, and in stressful environments, where a mother may have a higher number of offspring to ensure that at least some of them survive, lack of resources may impede their development due to increased competition for resources than smaller litters (Bautista et al, 2010). Larger litters generally have more competition for resources, such as maternal care and nutrients, leading to slower

developmental growth, which may include HPA activity and behavioural development. The consequence of competition itself may also be involved with the development of certain characteristics not experienced by individuals within small litters.

PNS has been associated with reduced litter size due to mild chronic stress and subsequent increase in body weight of offspring during adulthood in laboratory rats (Gotz, *et al*, 2008). They employed methods designed to reflect the effects of social stress, but without directly affecting maternal feeding behaviour in order to replicate a normal stressor in a naturalistic setting. A reduction in litter size, as seen in this study, provides increased accessibility to maternal milk and hence greater survival rates in the neonates (Gotz, *et al*, 2008). This study highlighted an adaptive mechanism triggered by mild PNS, evidenced by a lack of maternal biological indicators of chronic stress, such as a reduction in maternal body weight (Gotz, *et al*, 2008).

Litter size and its role in the development of play behaviour has been associated with a change in aggressive tendencies of the mother (Mendl, 1988). Comparisons between litters of two male kittens compared with single male kittens indicated that single kittens direct play towards their mother more than litters of two, however single kitten mothers show a higher level of aggression towards their young compare to mothers of litters of two (Mendl, 1988). Litter size has also been documented to be a factor in the onset of developmental milestones in a number of species (Rodel, 2010). Small litters of Long Evan laboratory rats have been reported

to have later onsets of motor ability directed at reaching central litter positions, compared to large litters (Bautista et al, 2010).

1.3.3. Sex specific effects of PNS

Male and female offspring have been shown to vary in their response to PNS (Bowman, *et al*, 2004; Kapoor, *et al*, 2006), however the cause of variation between male and females are currently not fully understood. Female rats born to mothers who have experienced PNS are more responsive to stress and have higher basal levels of HPA activity than those of male rats (McCormic, *et al*, 1995), who also suggests trans-generational influences. Bhatnagar et al (2005), however, demonstrated that PNS during the third trimester prevented HPA habituation during repeated handling stress in male adult offspring, suggesting that PNS may also influence the ability of males to manage chronic stress in some contexts. Bhatnagar et al, (2005) suggest the sex specific trait may be related to a habituation of the stress response specifically occurring in non-prenatally stressed male offspring. It has previously been noted that female rats show limited habituation and higher baseline levels of corticosteroids. As such, the inability of PNS male rats to habituate to mild stressors is more pronounced. Furthermore, Bhatnagar et al (2005) documented the involvement of testosterone, which is known to inhibit the stress response. PNS exposure during late gestation has been associated with reduced circulatory levels of testosterone within male offspring, which may also account for the generalised inability for female rats to habituate to repeated stress. Estrogens also stimulate ACTH production therefore fluctuations in gonadal hormones due to PNS may also be responsible for dysfunction in the stress response (Bhatnagar, et al, 2005). However, PNS in the last week of gestation of rats has been associated with

the masculinisation of female offspring. Del Cerro et al (2010) found that PNS actually decreased maternal oestradiol levels, causing male-like mitral cells within the accessory olfactory bulb (AOB) of female offspring (Bowman, et al, 2004; Del Cerro, et al, 2010).

The AOB receives input from the vomeronasal organ which detects pheromones. Mitral cells from the AOB project their axons to the amygdala and hypothalamus; these brain regions control behaviours such as aggressive and reproductive behaviours. Female rat pups, born to and reared by stressed mothers, (PNS included restraint, light and heat), have been documented as adults to exhibit aggressive behaviours towards their own pups (Del Cerro, *et al*, 2010). However, pups cross-fostered to non-stressed female dams, as adults display normal maternal behaviours towards their own pups, though hormonal and morphological neuronal changes remain (Del Cerro, *et al*, 2010). Similarly, female guinea pigs reared by PNS mothers also exhibit male like behaviours, which include play, courtship and social orientation (Sachser & Kaiser, 1996). PNS can therefore be linked to the exhibition of male like behaviour within female adults who were exposed to stress *in utero* (Perez-Laso et al, 2008; Green et al 2011).

1.4. Post-natal maternal environment and maternal deprivation

During early development, the developing brain is highly plastic, as such it can be easily influenced by environmental factors. The effect of maternal deprivation and early separation has been well studied and includes forced maternal separation such as early weaning, death of the mother and inadequate quality of maternal care induced by environmental influences and epigenetic changes.

Champagne & Curley (2009) documented the influence of maternal care on behavioural, physiological and epigenetic changes within the offspring of several species of mammal, in particular focussing on how limited maternal care can influence behavioural patterns and gene expression in the CNS. Maternal care itself can be influenced by a number of factors which include specific neuro-transmitters, hormones and PNS (Del Cerro et al, 2010).

The mother-infant relationship and sibling interactions within the post-natal period have been reviewed in some depth with regards to HPA activity, neuro-endocrine systems and social behaviour by Champagne & Curley (2009), who concluded that successive generations are found to reflect similar physiological and behavioural changes.

Disruption of maternal care and abnormal rearing experience in addition to PNS, also influence patterns of neuro-transmission in both the CNS and neuro-endocrine systems of the offspring. This can lead to a number of changes including the response to fear inducing stimuli, negative emotional states and behavioural attributes, in addition to reduced cognitive abilities (Flemming et al, 2002; Del Cerro et al, 2010).

Early research on maternal deprivation was conducted by Harlow and Suomi (1971) who investigated effect of social isolation of Rhesus macaques. They varied the degree of social and maternal deprivation between individuals during the first 6 months of life. Individuals that were fully isolated were unable to physically interact, see or hear other macaques during these six months. After this period, the macaques were studied in social housing, where behaviours were compared to individuals who had received normal maternal care. They found that individuals

who had been fully isolated during early life exhibited decreased cognitive function and had marked defects in behaviour. These included: reduced object exploration and restricted social behaviour such as reduced social approach, contact initiation, threatening behaviours and play behaviours; in addition to increased levels of disturbance related behaviours, such as rigid and passive posturing. Individuals also exhibited heightened fear responses when introduced to novelty as adults and exhibited stereotypic activities which included body rocking, bouncing and digit sucking (Harlow & Suomi, 1971).

Similarly, rodent pups who were deprived from complete maternal care during the post-partum period exhibit increased HPA activity, decreased exploratory behaviour and cognitive impairment as adults. These individuals also showed limited maternal care and behavioural attributes, namely deficits in hormonal priming of maternal behaviour and reduced maternal licking and grooming towards their own pups (Novakov & Flemming, 2005; Melo et al 2009). Such behavioural tendencies were also reported in subsequent generations as their offspring also showed limited maternal care (Flemming, et al, 2002; Kikusui et al, 2005).

Other studies have focused on prolonged periods of maternal and infant separation where behavioural defects include reduced social behaviour due to associated increase in HPA activity, though the extent varies with the experiment (Lehmann, & Feldon, 2000). Champagne and Curley (2009) referred to studies by Andrews and Rosenblum (1991, 1994), Coplan *et al* (1998, 2000, 2001, 2005) and Rosenblum *et al* (2001) who all documented the effects of such separation. In these studies, increased HPA activity as a direct result of forced separation was suggested as the cause of reduced expression of social behaviour within offspring, together with decreased levels of growth factors, reduced immunity and altered neuro-

transmitter metabolite levels in the anterior cingulate and medial temporal lobes; areas of the brain involved in perception of environmental cues and the initiation of behaviours linked to emotion.

In contrast to prolonged periods of separation or poor maternal care, it has been postulated that brief separation, such as handling of the dam for just 3-15 minutes, (rather than 3-4 hour separation as described above) during the pups' first few weeks of life stimulate maternal behaviours such as licking and grooming which increase the ability of the pups to handle stress as adults, particularly in pups derived from dams who normally exhibit low levels of maternal licking/grooming (Meaney, 2001). Rat pups who receive high levels of maternal licking and grooming are significantly less fearful of novelty compared to pups who receive limited maternal licking and grooming (Caldji, *et al*, 2000, Coutellier, *et al*, 2008). This corresponds to earlier work by Meaney *et al* (1989) who established that rat pups handled daily for the first three weeks of life demonstrate a greater efficiency to manage stress.

1.5. Epigenetic changes induced by altered maternal care

Epigenetic changes are described as environmental influences that change gene expression, namely the regulatory processes that allow for transcription of DNA. A potential example would be how the quality of maternal care may influence the development of young through differential gene expression. Changes in maternal care have been documented to have a direct influence on gene expression, which include RNA interference, chromatin remodelling, histone modification and DNA methylation (Champagne & Curley, 2009).

The intensity of maternal licking and grooming in rats has been reported to alter the expression of gluco-corticosteroid receptor genes within the hippocampus. A reduction in maternal care, such as lowered licking and grooming, lowers the number of gluco-corticosteroid receptors, which in turn inhibits HPA feedback loop, producing an increased secretion of glucocorticosteroids during a stressful event (Kikusui and Mori, 2009). Pups which have received elevated levels of maternal care have been found to have decreased methylation of the gluco-corticosteroid receptor 1_7 promoter. Such a decrease allows the promoter region to successfully allow transcription factors and RNA polymerase to access the DNA, which in turn produces an increase of gluco-corticosteroid receptors, promoting the negative feedback loop associated with normal HPA activity (Champagne & Curley, 2009).

Champagne & Curley (2009) describe the effect of acetylation - the process whereby an acetyl group (which has undergone an enzymatic reaction) attaches to the histone tail, which is normally associated with tight binding of DNA. The change of structure and polarisation of the histone tail by acetylation inhibits the histone's effect on the DNA structure – the DNA is less tightly coiled due to the two structures repelling one another thus allowing the DNA molecule to become exposed to the transcription factors, therefore more readily allowing gene expression. Increased methylation as described earlier also has a direct effect on acetylation. The increased production of protein complexes associated with methylation subsequently increase deacetylation. Such effects also inhibit gene expression, effectively silencing the gene (Champagne & Curley, 2009). Therefore, differences in gene expression as a result of such processes occur as the direct result of the levels and quality of maternal care received during the first weeks of life.

Kikusui and Mori (2003) documented disruption of interactions between mouse mothers and their pups and the effect of early weaning. They found that mice pups with poor maternal care exhibited increased anxiety and aggressive behaviours as adults in addition to higher HPA activity in response to novel stressors. Differences between male and female mice were also observed. Males were more vulnerable to early weaning stress than females; male mice showed increased myelination of the amygdala, specifically the anterior section of the basolateral amygdala alongside decreased neurochemical density within the hippocampus and prefrontal cortex (Kikusui and Mori 2003). It was concluded that the behavioural and neuro-chemical changes in adulthood were a result of the younger undeveloped brain's exposure to higher corticosteroid levels, due to the stress involved in early weaning during the late lactation phase (Kikusui and Mori 2009).

The phenomenon known as the maternal mediation hypothesis dictates that environment-dependent variables to which the dam is subjected in her environment are reflected in her behaviour towards her pups; assisting in the formulation of her offspring's phenotype (Smotherman & Bell, 1980). However, Marci and Wurbel (2006) suggest that such changes in phenotype cannot be a direct result of this hypothesis alone and suggest a new version that also includes the effect of direct environmental stressors on the offspring. Their reasoning is due to the conflicting research that opposed the traditional maternal mediation hypothesis. The hypothesis suggests that stress would inhibit maternal care, to produce offspring with an up-regulatory stress system and that this system would be of benefit to the offspring to prepare it for its future environment. However, Marci & Wurbel (2006) comment that small amounts of stress experienced by the mother increase maternal care towards the pups, leading to a down-regulation in stress reactivity. For example,

dams subjected to challenging situations or repeated mild stressors, such as moderately challenging foraging or brief handling are more attentive towards their young on return, producing pups who are able to develop a less reactive HPA system. However, mothers subjected to extreme adverse conditions such as long periods of maternal separation, produce pups who exhibit an increase in sensitivity of their stress system (Marci & Wurbel, 2006). Maternal separation has been associated with increased CRH mRNA in the paraventricular nucleus (PVN), increased corticosteroid response to stress and decreased levels of hippocampal GR mRNA, reduced exploratory movements and more general behavioural inhibition (Plotsky & Meaney, 1993; Meaney *et al*., 1995; Lehmann *et al*., 1999, Champagne and Curley 2009). Thus, suggesting that offspring that have no experience of stress or are subjected to severe stress both show an inhibition within their coping mechanisms as adults; this dysfunction causes HPA activity to become highly responsive. Whereas small amounts of repeated stress may better enable offspring to cope with stress as adults. They therefore suggest that the influence of environmental stress, which is facilitated by changes in maternal behaviour, affects development of the pups by means of epigenetic and neural changes (Marci & Wurbel, 2006). They provide a two-factor model to explain this; maternal care and environmental stress, rather than a linear single factor model (maternal care) due to their independent effects on the HPA development of the offspring.

Marci & Wurbel (2006) also state that experimental design in this expanding area of research must be standardised to control for environmental factors during trials, at present there is wide variation in experimental design in such studies. Another point raised is the rearing environment and its impact of the development of the offspring, suggesting that data may not be truly representative for species living

in a more naturalistic setting. As such Marci & Wurbel (2006) urge caution, when applying this hypothesis to other species, at least until more data is available. Such information is important when applied to the rearing context for other mammalian species. Adults with young offspring, raised in captivity and unable to cope with the environment may exhibit reduced maternal care, directly affecting the behavioural and physiological development of their young. Obviously, this is speculative and has yet to be fully investigated. Whereas induced brief periods of separation may in fact provide a positive effect on the behavioural development of offspring.

1.6. Sensitive periods in behavioural development and the effects of early weaning, socialisation and handling.

Early weaning occurs, by definition, at an age which is not natural to the species. A combination of cessation of nutritional factors and decreased social interactions can have an adverse effect. As described earlier, disruption of the mother-infant relationship of rodents can cause severe behavioural and physiological problems, namely an increased neuro-endocrine response. Early weaning can cause the offspring to become more sensitive to novelty, increased territorial aggression may be exhibited, and relationships between conspecifics are markedly unstable (Kikusui *et al*, 2007).

Early weaned pigs also exhibit behavioural inhibition, which could be considered as a welfare implication. Sumner et al (2008) weaned piglets at 12, 21 and 43 days, who were then habituated to an open field arena over a period of 6 days. Pigs were then subjected to a 5-min open-field test over three subsequent days; early weaned pigs exhibited reduced vocalisations and activity levels compared to individuals weaned at a later age (Sumner et al, 2008). Similarly, foals have also

been reported to exhibit weaning stress, specifically the development of stereotypic behaviours due to early weaning (Waran, *et al,* 2008).

It has also been documented that there is a gender difference in the response to early weaning, with male mice and rats reported to be more vulnerable to early weaning stress than females (Kikusui & Mori, 2009). However, such weaning practices have yet to be investigated fully within companion animals or equines (Waren, et al 2008).

Sensitive periods have been well documented in a range of species. Sensitive periods of development have been described as the specific time of individual's life in which certain types of association are learned as a result of external influences, enabling the animal to interact with its environment efficiently. Initially named critical period, it was later renamed sensitive period due to the discovery that this period had in fact a gradual and less defined onset and end than previously thought and was more flexible than originally proposed (Turner, 2004). Initial research by Lorenz (1937) was based on precocial species, e.g. ducklings, such species are well developed at their time of birth. His work on imprinting; development of the bond between the young of precocial species and its mother resulting in the young following the first moving object they see, initially coined the term "critical period". As a result, it was thought that this period was short and clearly defined chronologically. However, given our understanding of altricial species such as domestic cats, who are less flighty and slower to mature and so have the potential to exploit greater developmental plasticity during the early stages of life, this period of time in which factors have a marked effect on development is not so rigidly definable.

Sensitive periods vary between individuals of the same species and even between individuals of the same litter; namely due to genetic differences, variation within the physical environment, the individual's perception of the environment and variance of maternal care between individuals (Webster, 1997). The onset of a given sensitive period within an individual, as mentioned, is influenced by sensory and motor development, in addition to the animal's environment (Bateson, 1979), this in turn makes it difficult to determine an exact timescale at which a given period starts and finishes within a species. There is currently limited research on the sensitive periods for behavioural development in the domestic cat. However, Turner and Bateson (2000) have argued that the sensitive period for socialisation (rather than other sensitive periods, e.g. food preference) is between approximately 2 to 7 weeks of age.

Processes involved in early socialisation and handling of young have been well documented within many species, as research has focused within this field to facilitate a better relationship between human and animal and provide a higher level of animal welfare.

Socialisation is most effective when conducted within the related sensitive period of a particular species. However, the level at which socialisation influences behavioural development does depend upon genetic factors and changes to physiological functions before the sensitive period commences as discussed earlier.

McCune (1995) investigated the effect of socialisation and paternity on the development of cats' behaviour towards people and novel objects. She compared four test groups; friendly father/socialised, friendly fathers/unsocialised, unfriendly fathers/socialised and unfriendly fathers/unsocialised. Socialised kittens were

handled between 2 and 12 weeks of age, each litter received 5 hours of socialisation per week. Unsocialised kittens were only exposed to humans during cleaning and feeding routines. Kittens were then subjected to three experiments and behavioural response measured. She found that cats from a friendly father or who were socialised were quicker to approach a test person. Kittens from a friendlier father were also quicker to approach and be in contact with novel objects.

A similar study investigated the difference between standard socialisation (control group) and additional socialisation on kitten behavioural development. Individuals who received additional socialisation, which consisted of additional handling were found to exhibit fewer fear related behaviours and provided the owners a higher level of emotional support at one year of age when compared to the control group (Casey and Bradshaw, 2008).

It has been documented within other species that early socialisation between unfamiliar individuals improves welfare. Piglets, when socialised with unfamiliar individuals of the same species before weaning were more acceptable of individuals as aggression was reduced and social skills improved, leading to a reduction of aggressive behaviours (D'Eath, 2005). Early contact of piglets with humans also results in pigs at 18 weeks of age approaching and remaining in closer proximity to humans compared to those who were not handled at an early age (Hemsworth and Barnett, 2003). Similarly, cattle handled and hand fed during the first four days after birth were found to exhibit increased motivation to move towards a human compared to those who did not receive any handling/hand feeding (Krohn et al, 2001). Such intermittent separation is reflected in weaning practices at many cat charities across the UK, where over a two week period from 6 weeks of age, kittens

are taken away from the mother for a 30 minute period for health assessment and to mimic short term separation from the queen.

1.7. Implications and rationale of this study

Overall this chapter highlights the diversity of potential influences of genotype, prenatal maternal environment and post-natal environment on the physiological and behavioural development of animals including the offspring of domestic cats.

Regardless of mechanism, a better understanding of the relationship between these factors in the domestic cat would be beneficial to cats and the cat owning population. For example, identifying factors that lead to risks of delays or limitations in behavioural and physiological adaptations can lead to hypotheses that allow the development of interventions to moderate or avoid potentially harmful consequences. On the whole, studies of the influence of prenatal and post-natal challenge on behavioural development have been conducted under relatively controlled, laboratory conditions, including published studies of cat development. These studies therefore illustrate quite clearly some factors which *can* and *cannot* influence cat behavioural development, but not necessarily what *does* influence development in real world settings. Furthermore, maternal stress responses used as an indicator to determine individual phenotypic alterations within progeny are not apparent within the literature for many species including the domestic cat. Many studies have focused on specific stress treatments rather than maternal behaviour towards a stressor. This may be due to the need to define a clear distinction between what types of stressor influence major changes to neuroendocrine function within progeny of a species as a whole and their associated implications. However, as

individuals within a population react to stress differently, an investigation into the former may identify individuals or groups within a species who are more stress reactive.

In this project, therefore, we planned to focus on development of kittens in representative breeding conditions, in order to draw direct inference for that population. Inevitably such approaches will involve less control than experimental populations and the potential for multiple, confounding effects; our data collection depends on a survey of developmental milestones in kittens with a view to identifying factors of interest and their potential interactions, as a prerequisite for more controlled experimental studies. In common with many western nations, the majority of female cats of reproductive age in the UK have been neutered (Murray et al 2015) to avoid problems associated with unwanted kittens, although entire queen's are still be common in the free roaming, stray or feral cat populations. When pregnant queens join rescue or shelter populations, they are often allowed to progress to full term, with both the queens and their kittens neutered prior to rehoming. This study worked with Wood Green: The Animals Charity, who rehome about 1500 to 2000 cats per year including around 30-40 litters of kittens (Wood Green pers comm.). This represented a population of largely non-pedigree cats with notable variation in their response to carers. It was anticipated that this population as well as providing variation in response to captivity and close monitoring of kitten development by staff at the charity, would also provide opportunity for long term follow up of individual variation in behaviour following adoption, plus the potential to replicate studies over successive seasons or modify data collection as research questions were refined during the lifetime of the project. At the same time as

accessing shelter population of kittens, cat breeders were recruited to provide a comparative population of owned cats as well as provide further exploratory data with which to refine the direction of the project. Therefore. as well as objective data, such as age and breed of queen, the study also introduced a novel measure of temperament, based on a factor "queen's friendliness to humans" that should be identifiable to human carers, as well as a potential indicator of the queen's response to stressors during human care.

The aims of this initial study were to assess the variation in developmental milestones across these populations of kittens, identify factors that may influence these milestones, assess the feasibility of using owner/carer data, and determine additional data collection to validate and compliment owner/carer derived data. This first phase of the research relied on owner/carer recorded information and as a consequence chose measures that could be monitored as part of regular husbandry of the kittens without additional training or equipment. It was anticipated that further work that would have constituted the PhD element of this project would have included additional approaches, such as assessing the nutritional status and/or body condition of the queens, physiological measures of stress or coping such as corticosteroids and immunoglobulin measures, behavioural tests of the temperament or the queen and her kittens, and even where possible collecting data on the tom or toms that fathered the litters. These elements were not taken forward as part of this MSc by Research thesis, although then potential further work is presented and discussed as part of this thesis in the final chapter.

Chapter 2. Influence of maternal environment on kitten development: Introduction and methods.

2.1 Introduction

Although the average timing of various developmental stages in kittens has been extensively reported (e.g. see Bateson 2000 for review), the impact of maternal factors has received little attention, although her responses to environmental challenges before and after birth have the potential to influence the kittens' physical, physiological and psychological development. Approximately 20 studies have investigated personality in the domestic cat (reviewed by Gartner & Weiss, 2013), but few of these have investigated development of personality or temperament (e.g. Raihani, et al, 2014). The ability to predict individual behavioural outcomes in adult cats, and the mechanisms that would lead to differences in personality, such as learning capacity or emotionality, could improve care for kittens throughout the post-natal and juvenile period via modification of breeding and post-natal care practices.

The aim of the study was to investigate maternal factors that had the potential to influence the onset of anatomical and behavioural developmental stages of kittens. As of yet, causes for individual variation in development are not clearly understood and this is potentially a result of the highly complex relationship between multiple factors exerting their effect during an individual's lifetime. With this in mind, a study was undertaken in the domestic cat that comprises of an initial assessment of possible factors known in cats and other species to be influential on developmental milestones using carer/owner survey. Owner based surveys to obtain data from the real-life setting have been utilised more recently in companion animal

research. Owner based surveys have been utilised to report owner reported signs of lower urinary tract diseases in cats (Longstaff et al 2016), risk factors for road traffic collisions in cats (Wilson et al, 2016) and identification of early-life risk factors for feline overweight/obesity (Rowe et al 2015). The latter study relied upon owners recording specific information which included type, amount and frequency fed, alongside body condition score which required owners familiarising themselves with specific written instructions which led them to rate the body condition of their pet. With this in mind, a questionnaire was constructed to be consumer friendly, easy to complete with full instructions available for each carer. Validity and reliability of the questionnaire would have been undertaken during the next phase of the PhD.

2.1.1. Aims

The original aim was to complete this work as part of a PhD. Research presented in this thesis focused on a pilot study to assess the extent to which factors that may predict kitten development (identified from the literature and segregated into genetic, environmental and within litter variables) were related to the onset of developmental milestones.

Ongoing work would have included:

Direct observations of kittens to develop a detailed behavioural ethogram;

Validation and an assessment of the reliability of our queen temperament test, for example applying independent measures of temperament such as measures developed by Finka et al (2015).

Further rounds of modified surveys to compare pedigree vs unowned populations as a result of this initial work.

In this thesis, factors identified from a survey were subjected to statistical analysis to find out to what extent they predicted individual developmental milestones. The survey included a series of targeted observations to be undertaken by carers associated with specific developmental milestones, in order to identify what events appear most influential.

2.2. Materials and Methods

2.2.1. The Kitten Development Questionnaire

A kitten development questionnaire was created in order to capture data on behavioural and anatomical milestones in kittens from their human carers. The study was advertised by International Cat Care, Association of Pet Behaviour Councillors, the Governing Council of the Cat Fancy and "Your Cat" magazine. These were typically returned by breeders of pedigree cats (n = 28 litters). Data were also obtained from volunteer fosterers of Wood Green, the Animals Charity (n = 38), who cared for non-pedigree cats who were strays or had been relinquished. Respondents were asked to complete the questionnaire during the first 8 weeks post parturition. The survey consisted of three sections (see appendix A). Section one asked the owner to record details about the queen, tom (where known) and litter, such as sex, breed, age of queen and litter size. Section two was a record of when specific anatomical and behaviours stages were first witnessed within each individual within the litter and included the kittens' birth weight. The third section

used open ended questions to capture additional information surrounding the husbandry and housing of the queen and her kittens.

2.2.2. Survey overview

A number of maternal factors are thought to influence the behavioural and anatomical development of kittens, including queen's breed, age, weight, or body mass index (See Bateson 2000 for review). Six independent variables were derived for analysis relating to the nature of the queen and her litter. These were: the age of the queen at parturition; the queen's friendliness rating (QFR); the queen's breed; the number of kittens within the litter; the percentage of males within the litter and the birth weight of the kitten. These variables were selected either due to their known influence on certain developmental milestone from previous research; age of the queen, sex of the kitten were found to influence eye opening in kittens (Braastad and Heggelund 1984); breed differences and their influence on behavioural coping strategy (Marchei et al 2001); or their potential influence: friendly queens may be more accepting of human attention and thus early handling of kittens: early handling has been demonstrate to accelerate eye opening in kittens (Beaver 2003). Litter size has been documented as having differing effects on development in species other than the cat and was also worth considering as part of this study (Gotz et al, 2008; Bautistia 2010), as PNS (which may occur in under-socialised/unfriendly queens) has been documented to reduce litter size and increase birth weight.

Variables measured were also those that could be most readily obtained and recorded; recordings were kept as simple as possible to counteract variation in results by each observer, and to allow results to be more readily applied to the

general cat population. Early developmental milestones could be detected and recorded by the carer on the kittens' daily health check. Behavioural milestones relied upon the carers observing kittens which was part of the carer's daily tasks.

In this study we introduced a measure of personality which we defined as the queen's friendliness rating (QFR) which is an owner assessed measure of the queen's interaction with humans. To date there is not published reliable method to assess individual behavioural traits or personality in the cat, nor a viable yet easy way to measure stress reactivity which has been comprehensively certified (Bradshaw *et al*, 2012; Gartner & Weiss, 2013)). The QFR devised in this study used a Likert scale to rate "friendliness" (Fig 2.1), ranging from 1 – aggressive like tendencies and extreme nervousness towards people, to 9 – very friendly and affectionate towards people. This scale provided a general assessment of responsiveness to people by the queen, which may in turn be associated with underlying emotional parameters such as fear or stress reactivity, e.g. low QFR score would be indicative of low sociability towards a human caregiver and increased stress reactivity within the queen in the presence of humans. Such a scale may then provide more useful in the interpretation of results rather than using closed questions such as "is the queen aggressive". A measure of validation (external and convergent) and test reliability of the scale would have been undertaken before the next round of surveys. In particular it should noted that "friendliness" was a composite of at least 2 scales, one of involving aggressiveness and the other nervousness as the contrast to friendliness (Figure 2.1)

. Future work would have investigated if these could be better presented as separate scales as part of the validation of the approach.

Fig 2.1. Scale of queens' friendliness rating, taken from the kitten development form given to owners to complete.

Please indicate on the numerical scale below the temperament of your queen towards people								
1	2	3	4	5	6	7	8	9
Very aggressive or nervous			Shy		Approaches			Very friendly

2.2.3. Data Analysis - Statistical analysis

All litters could be used in analysis of developmental data although the total number of individuals per developmental stage differed due to missing data for a small number of litters for some weeks. List-wise option of analysis was chosen to compensate for missing data; as such the number of individuals per independent variable used within each analysis differs from the total number within the study.

Data analysis was conducted in SPSS19. Chi square and Kruskal-Wallis tests were initially used to investigate the effects of independent factors, on untransformed data such as breed and age classification on litter size, sex of kittens and birth weight, whilst Spearman rank analysis was used to assess if there were associations between factors such as litter size, sex ratio and body weight at birth.

To investigate the onset of each developmental milestone we applied parametric tests where data met the assumptions for parametric testing. Methods based on hierarchical regression; were adopted to assess the significance of factors,

their value as a tool to predict developmental timings and to pinpoint specific independent factors that affected the model. All factors were entered into the model using a forward method. As such all factors were taken into account by the hierarchical regression model; those which were found to be significant in the final step of the model are noted with their significance level in this report. Data inputted into the model first are those independent variables known to influence development in the cat and other species. Inputting breed first, controlled for this variable first in relation to following factors. For the regression models, independent variables were entered in the following order: Breed, age of the queen, litter size, percentage males within the litter, birth weight and finally the QFR. Residuals from the regression models were assessed for normality via a Kolmogorov-Smirnov test. Data that did not conform were transformed via ln transformation and re-entered into the regression model. Only when all assumptions were met to a satisfactory level was regression used. Assumptions include: no perfect multi-collinearity between independent variables (IV); IVs not correlated with factors not within the model; homoscedasticity of residuals; data is linear; errors are normally distributed; and that errors are also independent – tested by using a Durbin-Watson test (Field, 2005).

Some factors were regrouped for analysis; specifically data for litter size, queen's age and breed was re-categorised. Regrouping of the queens' age was specifically utilised as the majority of queens derived from the shelter population (the domestic cats) were either strays, as such a veterinary surgeon provided an approximate age of the queen, or the owners were not aware of the queen's date of birth. Litter size was grouped to alleviated issues that surrounded residual plots that would have deemed multiple regression unusable (Table 2.1), and was also congruent with previous studies (Marchei, et al, 2009).

Table 2.1. Independent variables categorised for the purpose of data analysis: values in parentheses indicate number of kittens for each category.

Independent variable	**Group**		
Queen's Age	Young queens ≤24months (163)	Older queens >24months (49)	
Litter size	Small litters ≤4 (136)	Large litters >4 115	
Breed	Domestic (149)	Western breeds (41)	Asian breeds (51)

As data were categorised in the hierarchical regression model, reference groups within categories were those who represented the majority; i.e. within-category groups (non-highlighted, Table 2.1) were compared to reference groups (highlighted, Table 2.1), therefore only non-highlighted groups were entered into the forwards entry hierarchical regression model. Numbers of individuals per category are detailed in Table 2.1. Birth weight and the QFR remained in their original format, sex ratio was analysed by percentage of males within the litter. The average for developmental onsets was calculated for all the kittens in this study.

Breeds were categorised into one of three groups, Domestic, Western and Asian (Table 2.2). Data were categorised in this way due to historic genetic links

between breeds and to previous work on breed differences and behavioural development in kittens (Marchei, *et al,* 2009). Data for the category of Australian Mist was omitted when analysing breed effects, due to the breed's lineage; this breed was created from only 30 foundation cats, 50% Burmese, 25% Abyssinian and 25% Domestic (Straede, n.d.).

Table 2.2. Composition of the three categories of breed.

Breed	**Total number of kittens**	**Number of litters**	**Re-grouped categories**
Domestic (moggy)	149	38	DOMESTIC
Maine coon	10	2	
Norwegian Forest	18	4	
Ragdoll	3	1	
Persian	2	2	WESTERN
British Short Hair	15	5	
Abyssinian	5	2	
Siamese	2	1	
Oriental	17	3	ASIAN
Birman	23	7	
Australian Mist	7	1	

Where developmental data did not meet assumptions for parametric analysis, appropriate non-parametric tests were used to assess the impact of each factor

separately. This included Kruskal-Wallis and Mann-Whitney U tests to test for differences between factors and Spearman rank test for correlations. As these involved a series of tests of each factor (as opposed to the combined testing of multiple factors afforded by hierarchical regression), we reported the significance of effects following the use of a sequential Bonferroni correction to correct for multiple comparisons. This allowed the most significant factors to be identified with some confidence, however, did not address issues such as lack of independence between factors, so findings of these tests should be treated with more caution than those from the hierarchical regression.

Chapter 3. Results of kitten development questionnaire

3.1. Overview of Sample Population

In total 66 questionnaires were returned with data on 251 kittens. 38 litters (149 kittens) had queens denoted as a Domestic (59.3%) who were all derived from the shelter population, whereas 28 litters (102 kittens) were born from queens that had been classed as a specific breed, all data from this group was obtained via specific cat breeders (40.7%). Descriptive statistics for litter size, birth weight, %males in litters and QFR are presented in Table 3.1. Litters of 3 (15 out of 66 or 23% of all litters), 4 (17/66 or 26%) and 5 (16/66 or 24%) were most common with litters of one kitten and 7 kittens only represented 3 times or 5% of population each. The majority of queens were given high scores on the QFR with 21 queens scored as 9, 16 queens scoring 8 queens and 12 scoring 7. Scores of 5 or less were only given to 10 queens in total, of which 5 scored 5, 4 scored 4 and only 1 queen was scored as 2.

Table 3.1 Descriptive statistics detailing averages for independent variable.

Independent variables	Pop	Mean	Std. Error	Std Deviation	Median	Range
Litter size		4.4	0.84	1.33	4	1-7
Birth weight (g)		114.9	2.42	31.86	110	40-202
% males		42.3	1.46	21.16	50	0-100
QFR		7.5	0.29	1.54	8	2-9

3.2. Factors influencing litter size, birth weight and sex ratio of litter.

There was no difference in birth weight of kittens between older and younger queens (Mann Whitney U = 2380, p = 0.68), nor was there any association between the age of queen and litter size (X^2 = 0.105, df = 1, p= 0.764). There was a significant effect of the age of queen and the percentage of males within litters (r_s = 0.161, p= 0.020), in addition to birth weight and litter size (r_s = -0.227, p= 0.003); as litter size increased, birth weight decreased (Figure 3.1). There was a borderline significance between size of litter and the percentage of males (r_s = -0.428, p= 0.051).

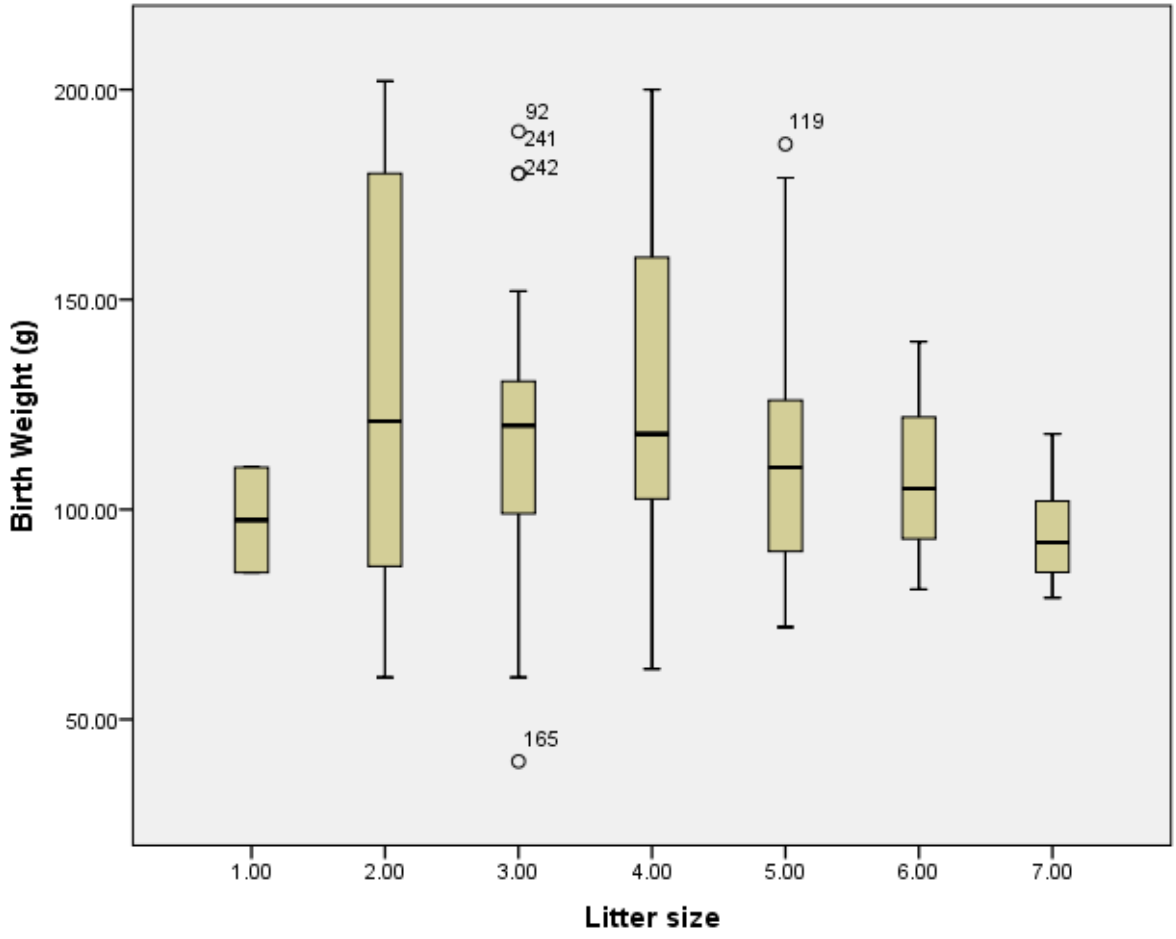


Figure 3.1. Birth weights and their relation to litter size.

The kittens' birth-weight varied significantly with breed (Kruskal-Wallis H2 = 55.58, p <0.000) (Figure 3.2). Post hoc tests with Bonferroni correction, revealed that kitten birth-weight was significantly lower in Asian breeds (Mdn = 90.16g) compared to Domestic (Mdn = 127.91g, Mann Whitney U = 379, p <0.000) and Western breeds (Mdn = 120.65g, Mann Whitney U = 237, p <0.000). However, there was no significant difference between Western and Domestic breeds (Mann Whitney U = 1657, p = 0.582).

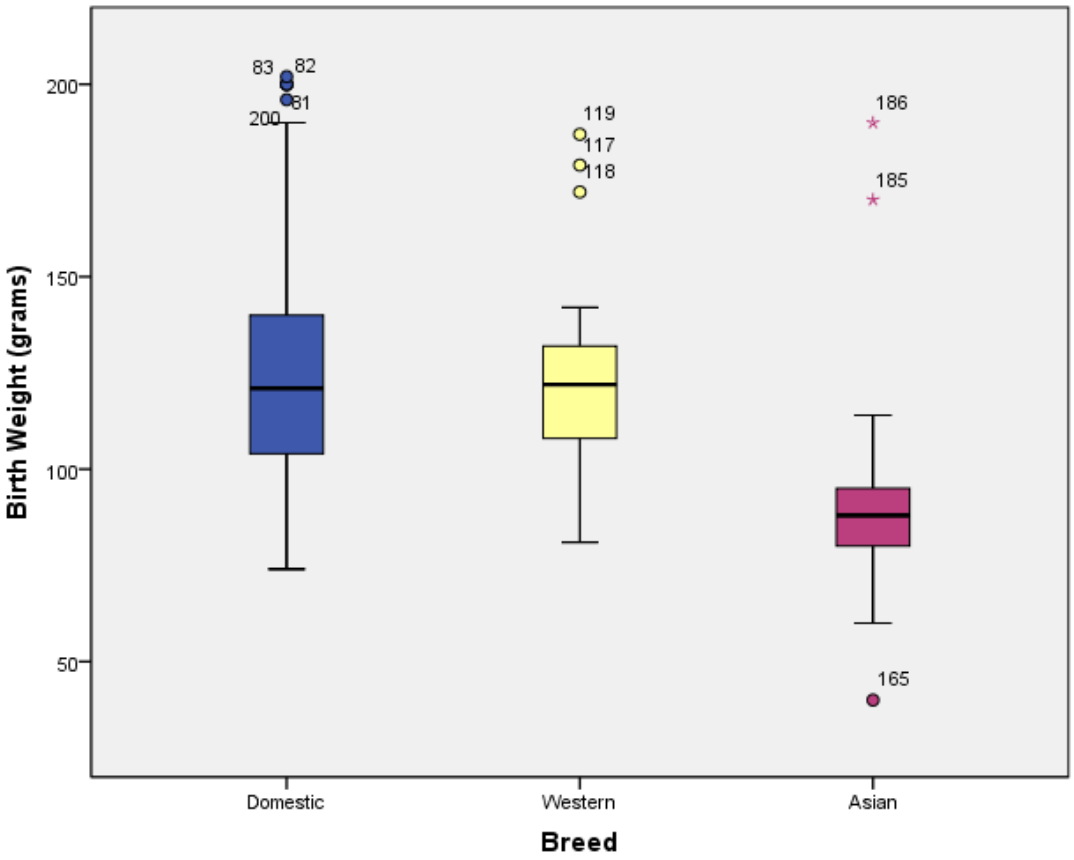


Figure 3.2 Birth weight of kittens from Domestic, Western and Asian breeds.

There was also a significant effect of breed on litter size (X^2 = 9.67, DF = 2, p = 0.008, Figure 3.3). Median litter size per breed are as follows; Asian (n= 46) 5; Western (n=46) 5; and Domestic (n = 149) 4.

Asian queens produced larger litters: 62.7% of Asian litters were classed as large; whereas Domestic queens tended to produce smaller litters with 63.8% litters classed as small.

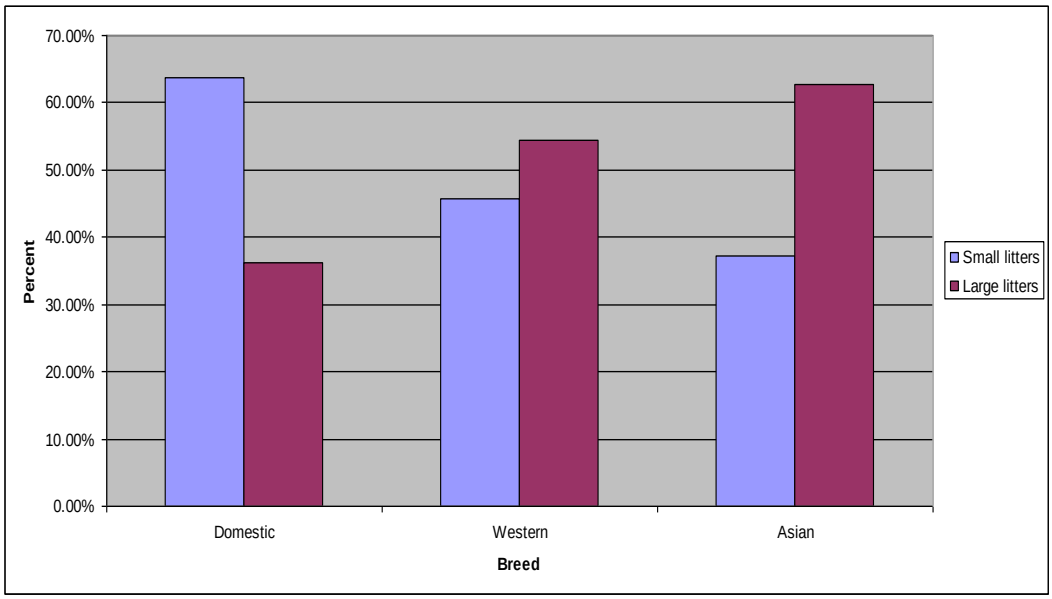
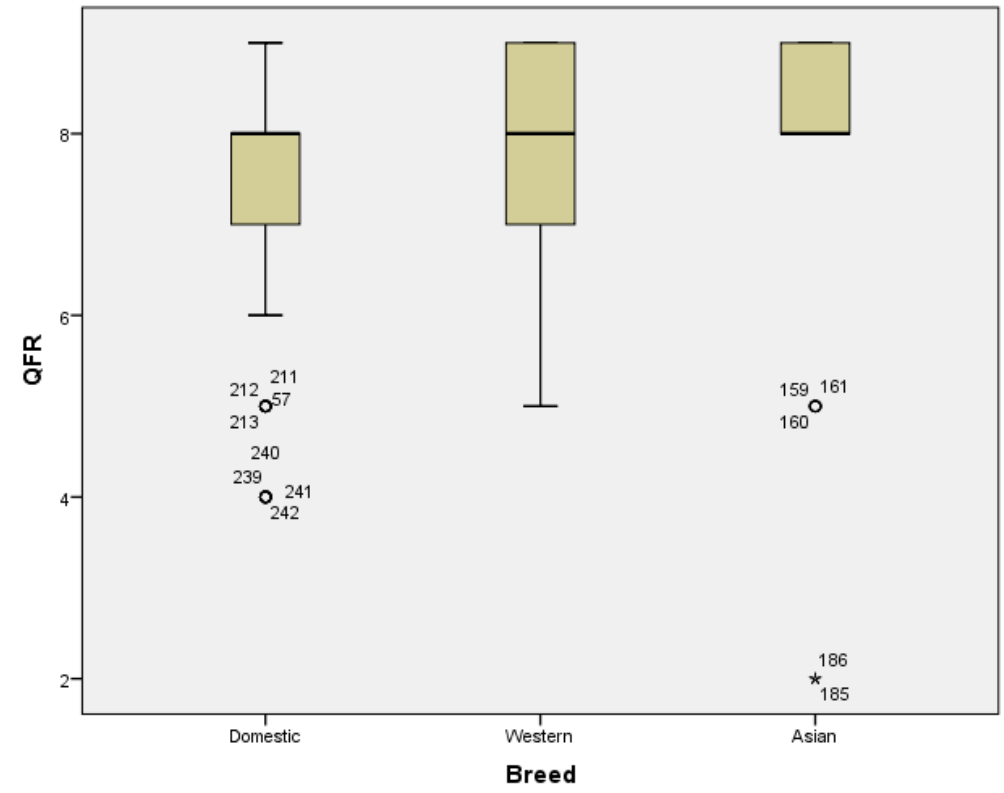
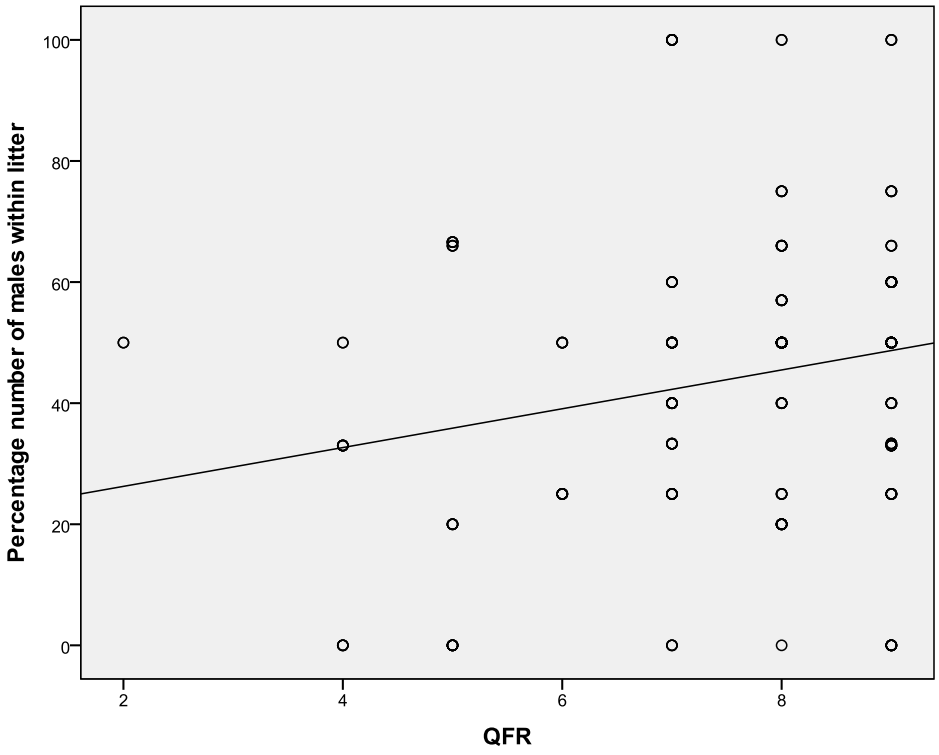


Figure 3.3. Percentage of Domestic, Western and Asian kittens with small litters (≤4) and large litters (>4).

No relationship was found between breed and percentage males within the litter (Kruskal-Wallis H = 4.409, p = 0.110), but there was a positive relationship between QFR and sex ratio within the litters (r_s = 0.150, p = 0.021, Figures 3.4 and 3.5). There was some evidence that low QFR may be associated with low litter size, with the one queen with 4 out of the 5 queens that scored 4 or less on QFR having smaller litters of 2 or 3 kittens, but the population of queens with low scores was too small to allow meaningful analysis. It was, however, found that breed was significantly associated with QFR (Kruskal-Wallis H = 15.473, p = 0.000). Using post hoc tests, results revealed that QFR was significantly lower in domestic (n = 141) compared to Western breed kittens (n= 46) (Mann Whitney U = 2342, p =

0.004), and Asian breed kittens (n = 39) (Mann Whitney U = 1845.4, p = 0.001), though not between Asian and Western breed kittens (Mann Whitney U = 855, p = 0.686).





Figures 3.4 and 3.5. Scatter and line graphs representing positive relationship between percentage number of males within the litter and the QFR (r_s = 0.150, p = 0.021).

3.3. Analysis of developmental onsets

The results section will present the summarised findings of the analysis as a table of the effects of each factor (Table 3.2) and figures showing the effects of litter size (Figure 3.6), breed of queen (Figure 3.7) and her age (Figure 3.8), followed by the findings of analysis of each milestone in turn.

Summary of Impact of different factors on development

Table 3.2 Significant factors contributing to the onset of anatomical and behavioural development in kittens

	Age of queen	Litter size	% Males in litter	Breed	QFR	Birth weight
Eyes opening [a]	B=-0.906 P < 0.001	B= -1.615 P < 0.001		B=-1.516 P < 0.001		B=-0.331 P < 0.001
Eyes open [b]		U=4907 P < 0.001	r_s =-0.220 p = 0.001	H_2=28.57 P<0.001		
Tooth eruption [c]	U=793.5 P = 0.004				r_s = -0.190, P = 0.015	
Moving from the nest [d]	U = 2465 P = 0.016	U = 4793.5 P < 0.001	r_s=-0.146 P = 0.026			r_s = -0.290, P < 0.001
Sibling play [e]	U = 2240.5, P = 0.012			H_2= 7.301 P < 0.001		
Self play behaviour [f]			r_s=-0.026, P < 0.001	H_2=31.503 P < 0.001		r_s = -0.484 P < 0.001
Eating solid food [† g]	B = 0.190 P = 0.038				B = -0.244 P < 0.001	
Object play behaviour [† h]		B = 0. 095 P < 0.05		B = 0.242, P < 0.001	B = 0. 036, P < 0.05	
Using the tray [i]		U = 4475.5 P = 0.019			r_s = -0.181, P = 0.011	

[†] Data LOG_e transformed.

[a] Kittens opened their eyes earlier when derived from older queens, large litter size, Asian breed and higher birth weights.

[b]Eyes becoming fully opened occurred earlier in kittens derived from large litter size, higher percentage of males within the litters and Asian breed.

[c,]Tooth eruption occurred earlier in kittens derived from young queens and queens' with a high friendliness rating.

[d]Kittens' moved from the nesting area at an earlier age when derived from young queens, small litter size, higher male sex bias within litter and higher birth weight.

[e]Sibling play occurred at an earlier age in kittens derived from young queens and domestic breed.

[f]Self play behaviours occurred at an earlier age in kittens derived from litters containing a high male sex bias, higher birth weight and in domestic and Western breeds.

[g] Solid food consumption occurred earlier in kittens derived from young queens with high QFR.

[h]Object play occurred at an earlier age in kittens derived from small litters, Domestic and Western breed, and kittens derived from unfriendly queens.

[I]Tray usage occurred earlier in kittens derived from larger litters and queens' with a high QFR.

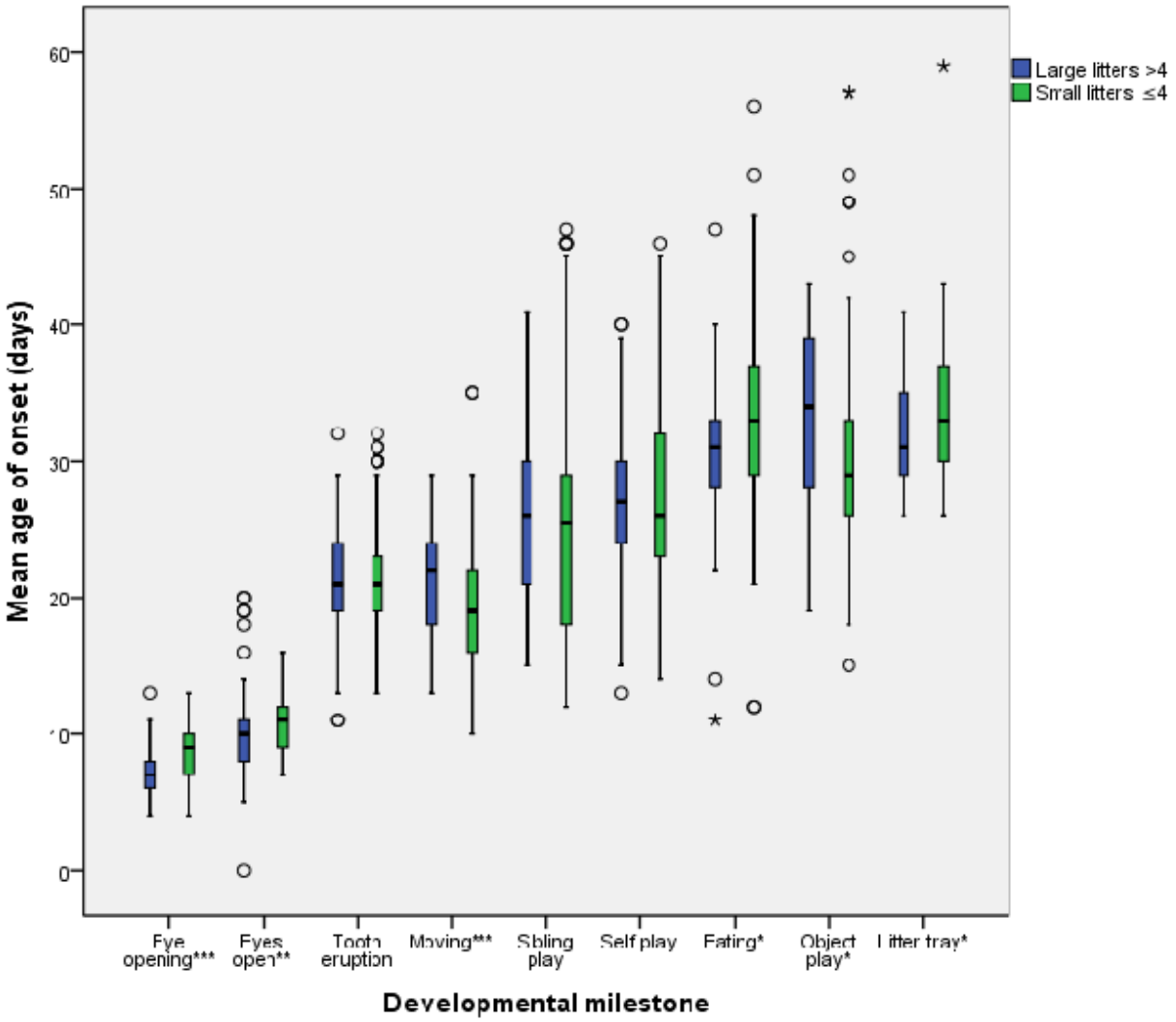


Figure 3.6 Comparison of age of development (mean value) between small litters (≤4) and large litters (>4). *p<0.05; **p<0.005; ***p<0.001.

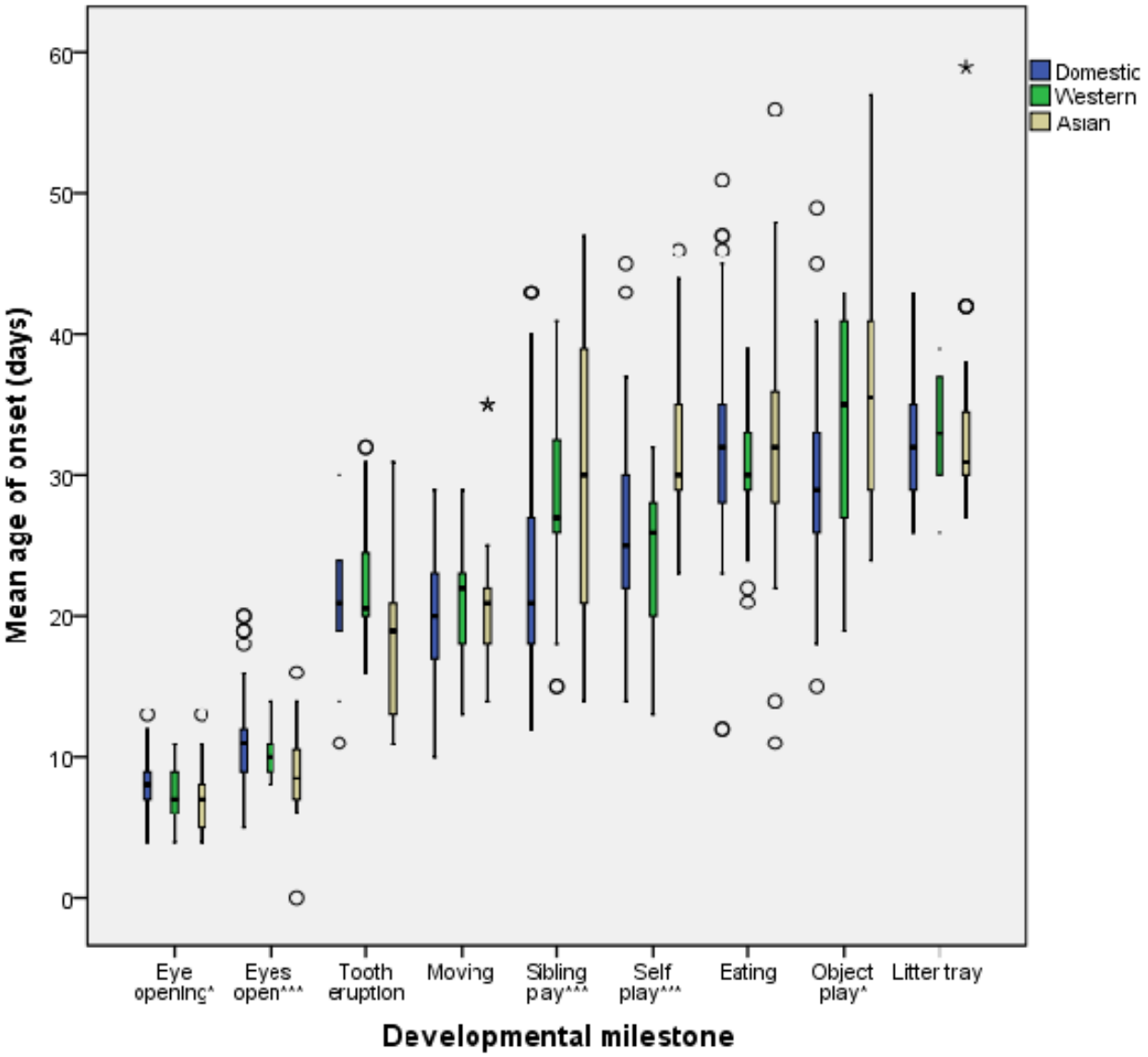


Figure 3.7 Comparison of age of development (mean value) between breed type (Domestic, Western and Asian). *p<0.05; ***p<0.001.

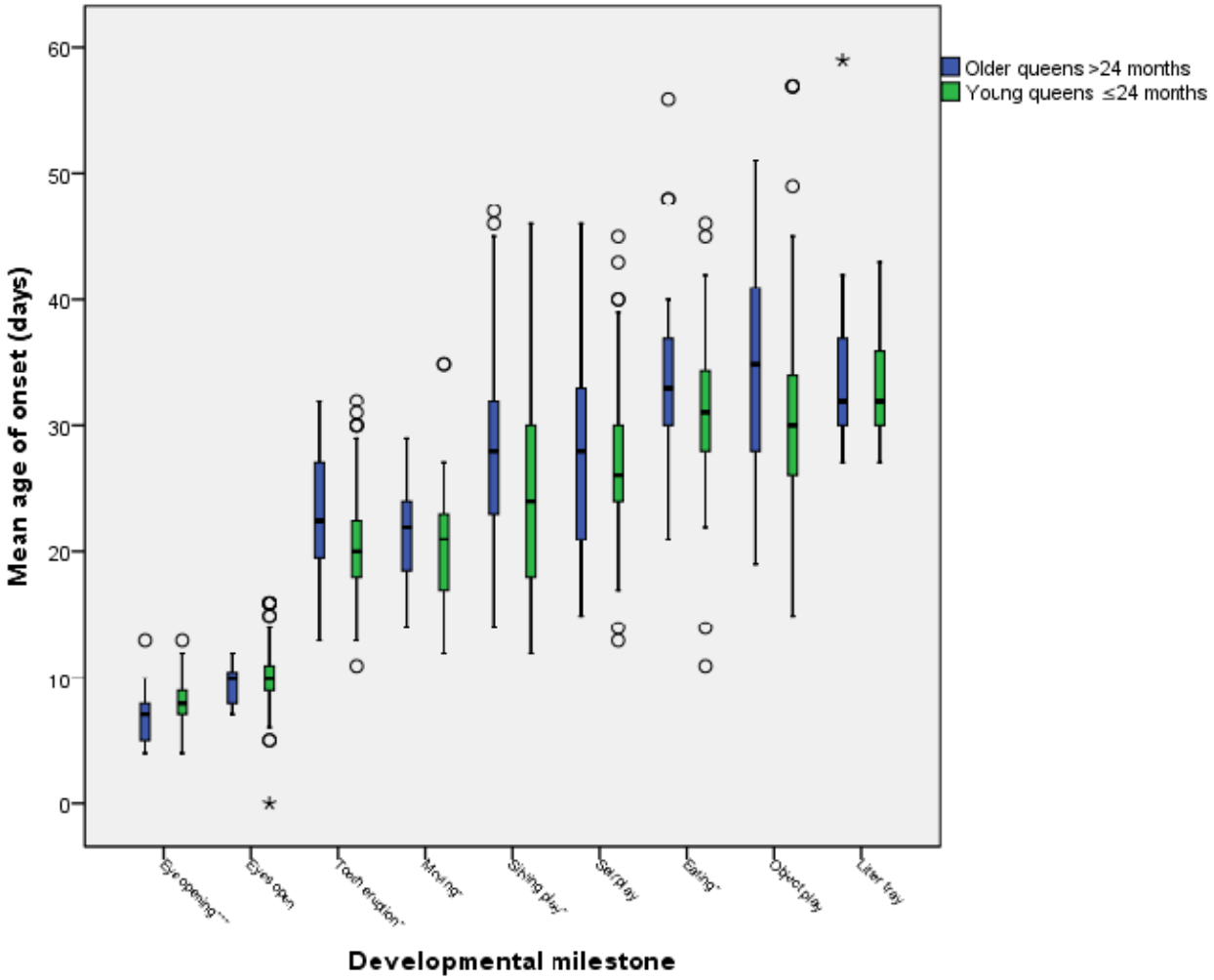


Figure 3.8 Comparison of age of development (mean value) between young queens ≤24 months and older queens >24 months. *p<0.05; **p<0.005; ***p<0.001.

3.3.1. Eye Opening

Eye opening was recorded as being the appearance of a slit within at least one eye. The average for eye opening for the whole population (n = 211) was defined as 7.85 days (*SE* 0.198).

When predicting the onset of eye opening, implementing forward hierarchical regression, a significant model emerged ($F(124) = 13.07$, $p < 0.001$, $r2 = 0.351$; see Table 3.3 for summary statistics and Table 3.4 for results of hierarchical regression). Significant contributing factors were:

1) Breed; Asian kittens opened their eyes at an earlier age than Domestic kittens ($B = -1.516$, $p < 0.001$), whereas there was no difference between Western and Domestic kittens.
2) Age of the queen; kittens from older queens open their eyes significantly earlier than those derived from young queens ($B = -.906$, $p = 0.003$).
3) Litter size; kittens from a larger litter size (5 or more individuals) opened their eyes at an earlier age ($B = -1.615$, $p < 0.000$), compared to small litters (≤4 individuals).
4) Birth-weight; as birth weight increased time of eye opening decreased ($B = -.331$, $p < 0.000$), see also Figure 3.9.

Table 3.3. Descriptive statistics. Average onset of eye opening for young (≤24 months) and old queens (>24 months), small (≤4 individuals) and large litters (>4 individuals) and three breed categories. Data includes number of cats for which data was reported, mean, SE and SD of number of days, median number of days since birth and IQR.

		N	Age (days mean	Age (days Std. Error	Std Deviation	Median	IQR
Age of queen	Young mothers	138	7.89	0.174	1.96	8	2
	Older mothers	32	6.81	0.58	1.788	7	3
Litter Size	Small	99	8.38	0.193	1.920	9	2
	Large	71	6.72	0.225	1.899	7	3
Breed	Domestic	97	8.24	0.192	1.892	8	2
	Western	30	7.17	0.343	1.887	7	2
	Asian	43	6.81	0.343	2.249	7	3

Table 3.4. Hierarchical regression (final step –forwards mode) for eyes opening

Eyes Opening	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta
(Constant)	10.638	.631	
Asian	-1.516	.337	-.377***
Older Queens	-.906	.303	-.221**
Large Litters	-1.615	.264	-.457***
BW	-.018	.004	-.331***

Note $\Delta R^2 = 0.083$ for step 4; **p<0.005; ***p<0.001.

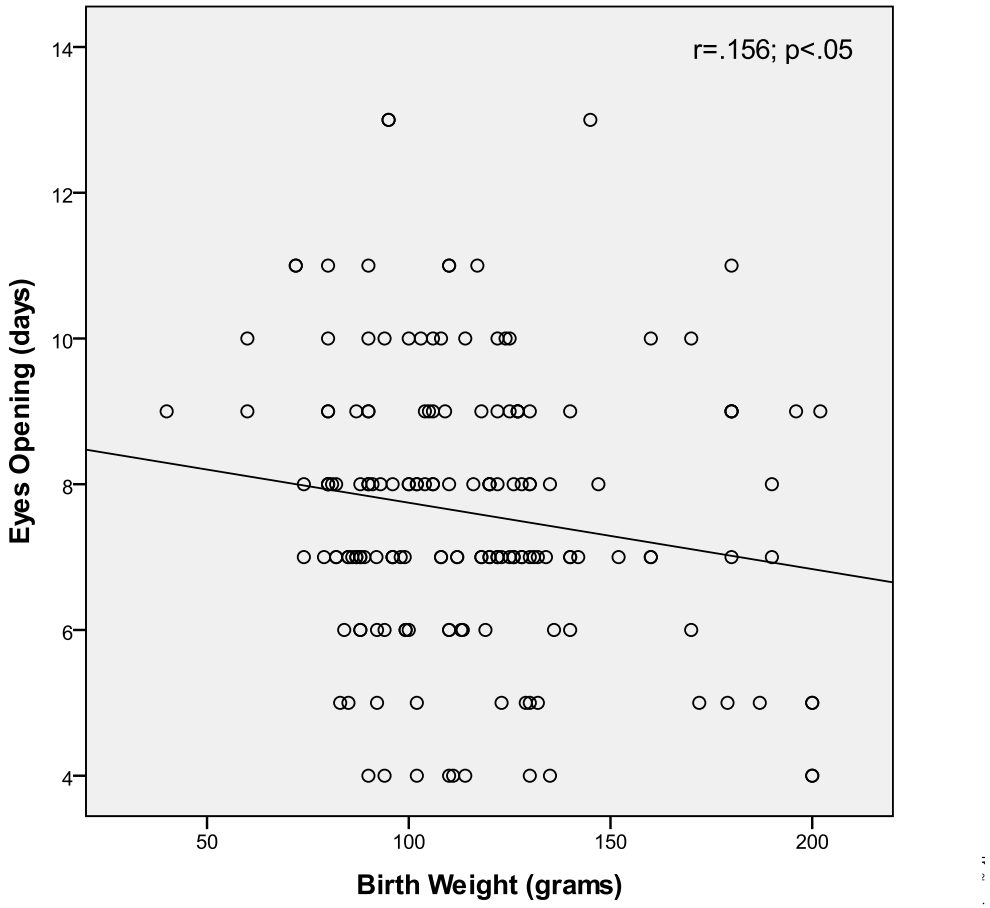


Figure 3.9. Scatter and line graph representing a negative relationship (r^2 0.024) between individual birth weight (grams) and time at which eyes open (days).

3.3.2. Eyes Fully Open

Time of eyes fully open was measured by both eyes being completely open. The average age for eyes being fully open for the whole population (n = 232) was defined as 10.43 days (*SE* 0.175).

Data did not meet assumptions for regression so non-parametric tests were adopted. A significant effect of percentage male on time of eyes fully open was found (r_s = -0.220, p = 0.001). There was also a significant effect of queen's breed on time of eyes being fully open (Kruskal-Wallis H2 = 28.57, p = 0.000). Post-hoc tests revealed (using a Bonferroni correction, value of 0.017) that Domestic kittens fully

opened their eyes later than Western (Mann Whitney U = 2705, p = 0.000), as did Asian breeds (Mann Whitney U = 863, p = 0.006). Kittens from small litters fully opened their eyes later than kittens derived from large litters (Mann Whitney U = 4907, p = 0.000). There was no relationship between eyes being fully open and birth weight (r_s = -0.069, p = 0.380), or QFR (r_s = -0.012, p = 0.854) or age of queen (Mann Whitney U = 2547, p = 0.111).

Table 3.5. Descriptive statistics. Average onset of eye fully open for young (≤24 months) and old queens (>24 months), small (≤4 individuals) and large litters (>4 individuals) and three breed categories. Data includes number of cats for which data was reported, mean, SE and SD of number of days, median number of days since birth and IQR.

Eyes Fully Open		N	Age (days)		Std Deviation	Median	IQR
			Mean	Std. Error			
Age of queen	Young mothers	146	10.10	0.215	2.602	10	2
	Older mothers	32	9.38	0.241	1.362	9	3
Litter Size	Small	101	10.72	0.212	2.127	10	3
	Large	77	8.99	0.283	2.484	9	4
Breed	Domestic	103	10.56	0.219	2.221	10	3
	Western	31	9.81	0.302	1.982	9	3
	Asian	44	8.70	0.434	2.882	8.5	4

3.3.3.Tooth Eruption

Time of tooth eruption was measured by the appearance of any tooth emerging from the gum-line. The mean age for tooth eruption for the whole population (n = 174) was defined as 21.11 days (*SE* 0.315).

Data did not meet assumptions for parametric regression so non-parametric tests were used. Date suggested tooth eruption occurring earlier in Asian breeds compared to both Western and Domestic kittens but this was not significant (Kruskal-Wallis $H_2 = 5.896$, $p = 0.052$). Tooth eruption was earlier in kittens from young queens then older queens (Mann Whitney $U = 793.5$, $p = 0.004$). There were a lack in significant effects of litter size (Mann Whitney $U = 3319$, $p = 0.299$), birth weight ($r = 0.062$, $p = 0.507$) or percentage males ($r_s = 0.100$, $p = 0.189$) on age of tooth eruption, though there was a negative relationship between tooth eruption and QFR ($r_s = -0.190$, $p = 0.015$).

Table 3.6. Descriptive statistics. Average for tooth eruption for young (≤24 months) and old queens (>24 months), small (≤4 individuals) and large litters (>4 individuals) and three main breed categories. Data includes number of cats for which data was reported, mean, SE and SD of number of days, median number of days since birth and IQR.

Tooth eruption		N	Age (days) Mean	Std. Error	Std Deviation	Median	IQR
Queen age	Young mothers	113	20.27	0.385	4.091	20	4
	Older mothers	21	24.1	1.097	5.029	21	11
Litter Size	Small	86	21.43	0.448	4.152	21	4
	Large	48	19.88	0.698	4.836	19	4
Breed	Domestic	89	21.08	0.388	3.659	21	5
	Western	16	22.38	1.132	4.530	21	6
	Asian	29	19.41	1.131	6.127	19	9

3.3.4. Kittens' Moving from Nest

Kittens' age of moving from the nesting area was measured from when the kittens were observed to move out of the immediate nesting area to explore their surroundings (i.e. the queen's bedding area), which may include following the queen. The mean age for kitten moving for the whole population (n = 232) was defined as 20.09 days (*SE* 0.315).

Differences were found between older and younger queens, and the timing of kitten movement from the nest (Mann Whitney U = 2465, p = 0.016). Kittens from large litters exhibited movement at a later age compared to small litters (Mann Whitney U = 4793.5, p = 0.000). Percentage males within litter was associated with timing of kitten movement (r_s = -0.146, p = 0.026) and a relationship was found between birth weight and timing of kitten movement (r_s = -0.290, p = 0.000) (Figure 3.10). However, kittens moving from the nest and breed did not produce a significant result (Kruskal-Wallis H2 = 2.572, p = 0.276).

Table 3.7. Descriptive statistics. Average time at which the kittens moved out of the immediate nesting area to explore for young (≤24 months) and old queens (>24 months), small (≤4 individuals) and large litters (>4 individuals) and three main breed categories. Data includes number of cats for which data was reported, mean, SE and SD of number of days, median number of days since birth and IQR.

Kittens moving from nest		N	Age (days) Mean	Std. Error	Std Deviation	Median	IQR
Age of Queen	Young mothers	145	19.71	0.420	5.058	21	6
	Older mothers	35	21.11	0.657	3.886	21	6
Litter Size	Small	104	19.26	0.56	4.706	20	8
	Large	76	20.97	0.368	3.204	22	4
Breed	Domestic	104	19.24	0.506	4.164	20	7
	Western	33	21.06	0.733	4.213	22	6
	Asian	43	21.95	0.666	4.370	21	4

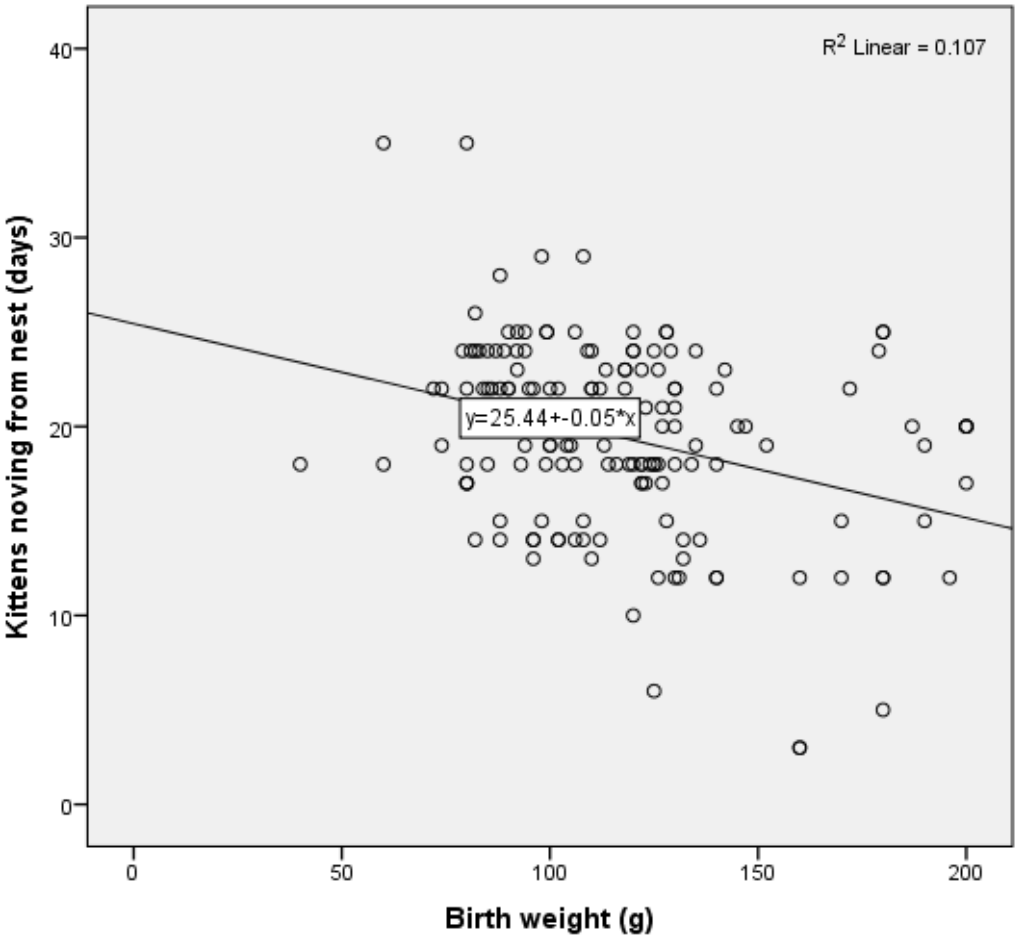


Figure 3.10. Scatter and line graph representing the negative relationship ($r^2 = 0.11$) between day when kittens were first noticed moving from the nest (days) and the queens' friendliness rating.

3.3.5. Play with Litter-Mates

Play with littermates was recorded when the kitten was interacting and/or imitating play behaviours with siblings. Mean age for play with litter mates for the population (n=231) as a whole was 25.67 days (*SE* 0.516).

The onset of sibling play was significantly later in kittens derived from older queens (Mann Whitney U = 2240.5, p = 0.012). Litter size exhibited a trend towards influencing the onset of sibling play (Mann Whitney U = 5662, p = 0.055). There

was no significant difference with the percentage of males within the litter (r_s = -0.066, p = 0.316).

Queens' breed did predict timing of sibling play behaviour (Kruskal-Wallis H2 = 27.301, p = .0000). Using post hoc tests, results revealed that the onset of behaviour significantly occurred earlier in domestic kittens compared to Western breed kittens (Mann Whitney U = 1581.5, p = 0.000), and Asian breed kittens (Mann Whitney U = 1709, p = 0.000), though not between Asian and Western breed kittens (Mann Whitney U = 614, p = 0.114). There were no significant correlations between the onset of sibling play and birth weight (r_s = -0.123, p=.118) or QFR (r_s=0.035, p=0.606).

Table 3.8 Descriptive statistics. Average for time at which the kittens exhibited play with siblings for young (≤24 months) and old queens (>24 months), small (≤4 individuals) and large litters (>4 individuals) and three main breed categories. Data includes number of cats for which data was reported, mean, SE and SD of number of days, median number of days since birth and IQR.

Play Litter Mates		N	Age (days) Mean	Std. Error	Std Deviation	Median	IQR
Age of Queen	Young mothers	141	24.99	0.707	8.395	25	12
	Older mothers	34	28.15	1.528	8.911	26.5	11
Litter Size	Small	99	24.88	0.913	9.079	23	11
	Large	76	26.54	0.895	7.800	25.5	14
Breed	Domestic	100	22.57	0.685	6.848	21	9
	Western	32	28.03	1.287	7.28	28	13
	Asian	43	30.84	1.523	9.986	30	18

3.3.6. Self Play

Self play was recorded as the time at which the kitten was witnessed playing alone. Mean age for self play for the population (n=226) as a whole was 27.19 days (*SE* 0.425).

The onset of self play behaviours and 1) kittens derived from older and young queens (Mann Whitney U = 2897, p = 0.463), 2) between kittens derived from small or large litters (Mann Whitney U = 6325, p = 0.943) did not yield any significant results. Yet breed differences were found (Kruskal-Wallis H2 = 31.503, p = 0.00). Post-hoc analysis suggests that onset of self play occurred in Asians at a later age than both domestic (Mann Whitney U = 1472.5, p=0.000) and Western kittens (Mann Whitney U = 248, p = 0.000). The onset of self play behaviours was associated with the percentage of males within the litter (r_s = -0.265, p < 0.001). Birth weight and the onset of self play behaviours exhibited a significant negative correlation (r_s = -0.484, p = 0.000) (Figure 3.11). Though self play behaviour and the QFR were not of great significance (r_s = 0.38, p = 0.585).

Table 3.9. Descriptive statistics. Average time at which the kittens exhibited self play for young (≤24 months) and old queens (>24 months), small (≤4 individuals) and large litters (>4 individuals) and three main breed categories. Data includes number of cats for which data was reported, mean, SE and SD of number of days, median number of days since birth and IQR.

Self play		N	Age (days) Mean	Std. Error	Std Deviation	Median	IQR
Age of Queen	Young mothers	143	27.04	0.492	5.878	26	6
	Older mothers	34	26.29	1.381	8.055	27	12
Litter Size	Small	101	27.5	0.653	6.564	26	9
	Large	76	26.11	0.684	5.966	26.5	7
Breed	Domestic	102	25.87	0.582	5.883	25	7
	Western	32	23.84	0.897	5.074	25	9
	Asian	43	31.6	0.884	5.795	30	6

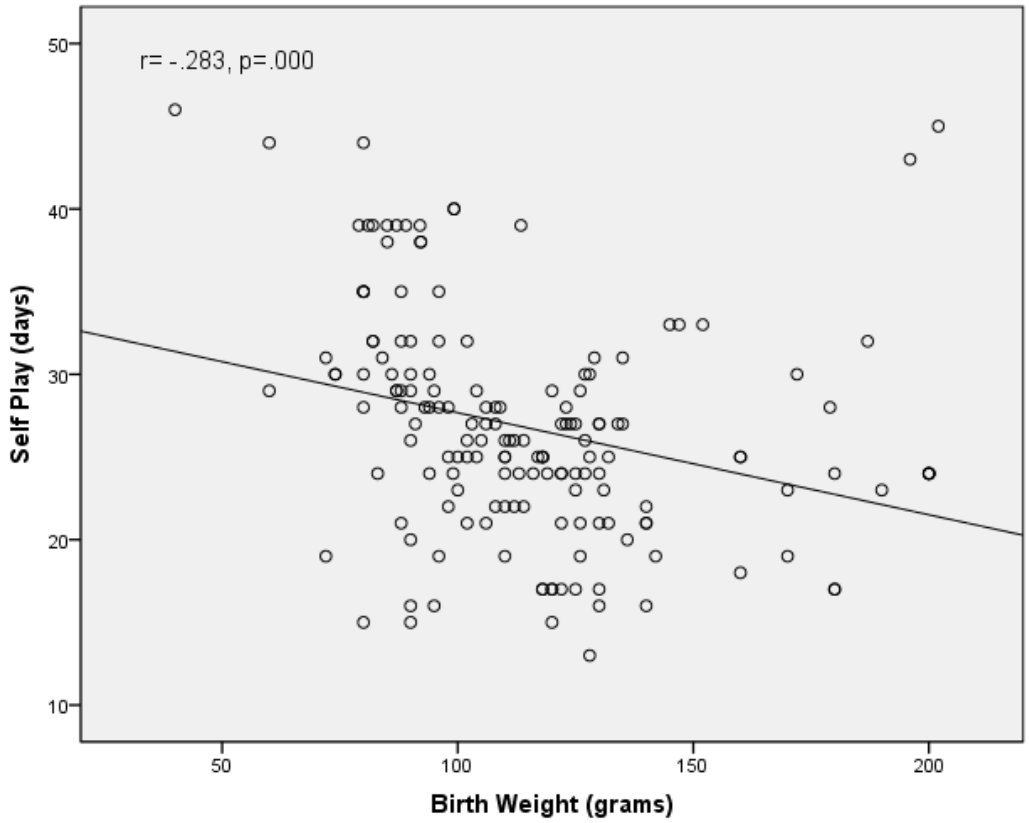


Figure 3.11. Scatter and line graph representing a negative relationship (r^2 = 0.08) between self play behaviour (days) and birth weight (grams).

3.3.7. Eating solid food

Solid food ingestion was measured when the kitten was witnessed eating food provided by the observer. Mean age for solid food ingestion for the population (n=221) as a whole was 31.90 days (*SE* 0.403).

Data for timing of solid food ingestion was Ln transformed before entering into the regression model. When predicting timing of solid food consumption a significant model emerged ($F(123) = 4.238$, $p = 0.001$, $R^2 = 0.152$) (Table 3.11). Factors contributing to the time of solid food consumption were

1) Age of the Queen, older queens had kittens with a later onset of food consumption (B = 0.190, p = 0.038),

2) The QFR; mothers with high QFR produce kittens who demonstrated eating solid food at an earlier age, compared to kitten from queens with a lower QFR (B = -0.244, p <0.006) (Figure 3.12).

Table 3.10 Descriptive statistics. Average onset of solid food ingestion for young (≤24 months) and old queens (>24 months), small (≤4 individuals) and large litters (>4 individuals) and three main breed categories. Data includes number of cats for which data was reported, mean, SE and SD of number of days, median number of days since birth and IQR.

Eating solid food		N	Age (days)		Std Deviation	Median	IQR
			Mean	Std. Error			
Age of queen	Young mothers	141	31.23	0.455	5.339	31	7
	Older mothers	34	34.24	1.232	7.182	33.5	8
Litter Size	Small	104	33.36	0.58	5.917	33	9
	Large	71	29.55	0.604	5.090	30	5
Breed	Domestic	105	32.23	0.494	5.058	32	8
	Western	32	30.91	0.769	4.35	30	5
	Asian	38	31.42	1.394	8.595	32	8

Table 3.11 Hierarchical regression (forwards model) for the onset of eating solid food

Eating solids	Unstandardized Coefficients		Standardized Coefficients
	B	Std. Error	Beta
(Constant)	3.648	.073	
Asian	.062	.038	0.147
Western	-.019	.039	-0.048
Older Queens	.075	.036	0.190*
Large Litters	-.057	.031	-.160
QFR	-.027	.010	-.244***

Note $\Delta R^2 = 0.808$ for step 4. *p<0.05, ***p<0.001.

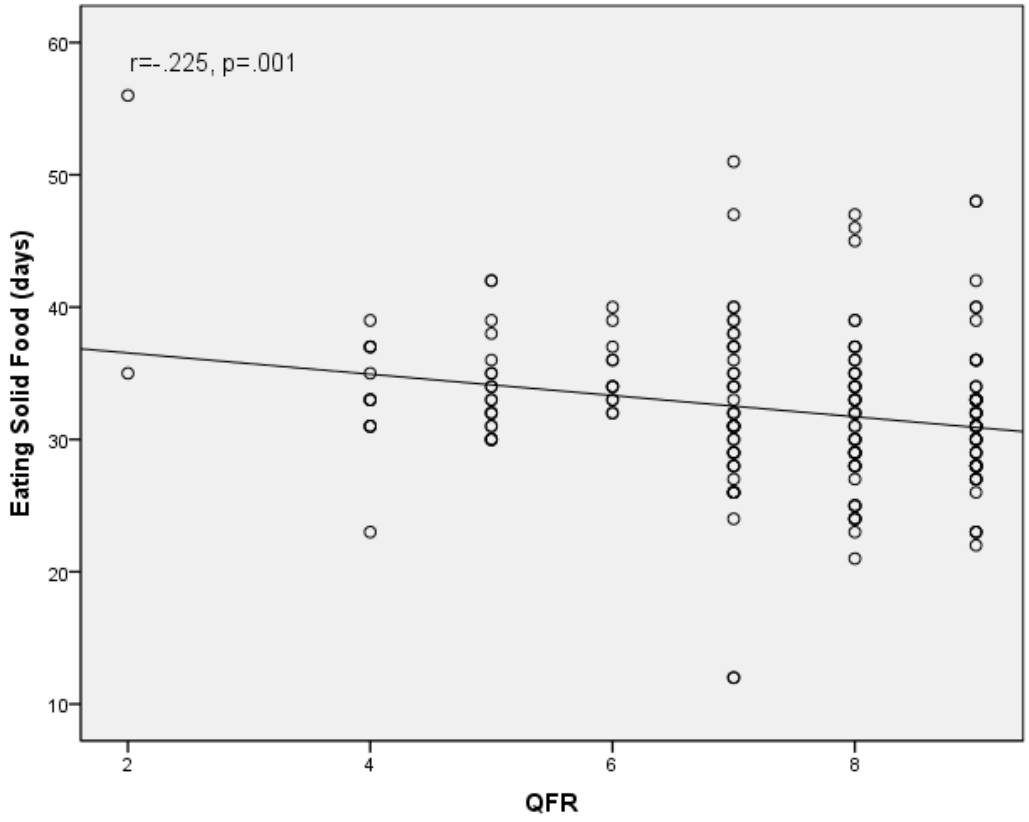


Figure 3.12. Scatter and line graph representing a negative relationship ($r^2 = 0.05$) between day when solid food consumption started (days) and the queens' friendliness rating.

3.3.8. Object play

Object play was recorded as the date at which the observer witnessed the kitten playing with an object, such as a ball, piece of string etc, for the first time. Mean age for object play for the population (n=209) was 31.91 days (*SE* 0.500).

Data for timing of object play was Ln transformed before entering into the regression model. When predicting timing of onset of object play a significant model emerged ($F(115) = 11.406$, $p = 0.000$, $R^2 = 0.291$) (Table 3.13). Significant factors related to the onset of object play behaviours were

1) Breed; kittens derived from Asian breeds ($B = 0.242$, $p < .000$) had a later onset compared to domestic kittens.
2) Litter Size: Kittens from a large litter had a later onset of object play behaviour compared to kittens from small litters ($B = 0.095$, $p < .05$).
3) QFR: Kittens from friendly queens also showed a later onset of play behaviour ($B = 0.036$, $p < .05$).

Table 3.12. Descriptive statistics. Average time at which the kittens exhibited object play for young (≤24 months) and old queens (>24 months), small (≤4 individuals) and large litters (>4 individuals) and three main breed categories. Data includes number of cats for which data was reported, mean, SE and SD of number of days, median number of days since birth and IQR.

Object play		N	Age (days) Mean	Std. Error	Std Deviation	Median	IQR
Age of Queen	Young mothers	130	31.05	0.638	7.278	30	8
	Older mothers	34	33.74	1.421	8.288	34	13
Litter Size	Small	93	30.85	0.863	8.325	28	7
	Large	71	32.59	0.750	6.321	33	9
Breed	Domestic	94	29.86	0.533	5.167	28	7
	Western	32	33.66	1.413	7.994	36.5	16
	Asian	38	36.66	1.465	9.032	35.5	12

Table 3.13. Hierarchical regression (final step –forwards model, entry method) for object play

Object Play	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta
(Constant)	3.193	.150	
Asian	.242	.049	.426***
Western	.086	.047	.159
Large Litters	.095	.040	.197*
QFR	.036	.013	.226*

Note $\Delta R^2 = 0.038$ for step 4. ***p<0.001, **p<0.005, *p<0.05.

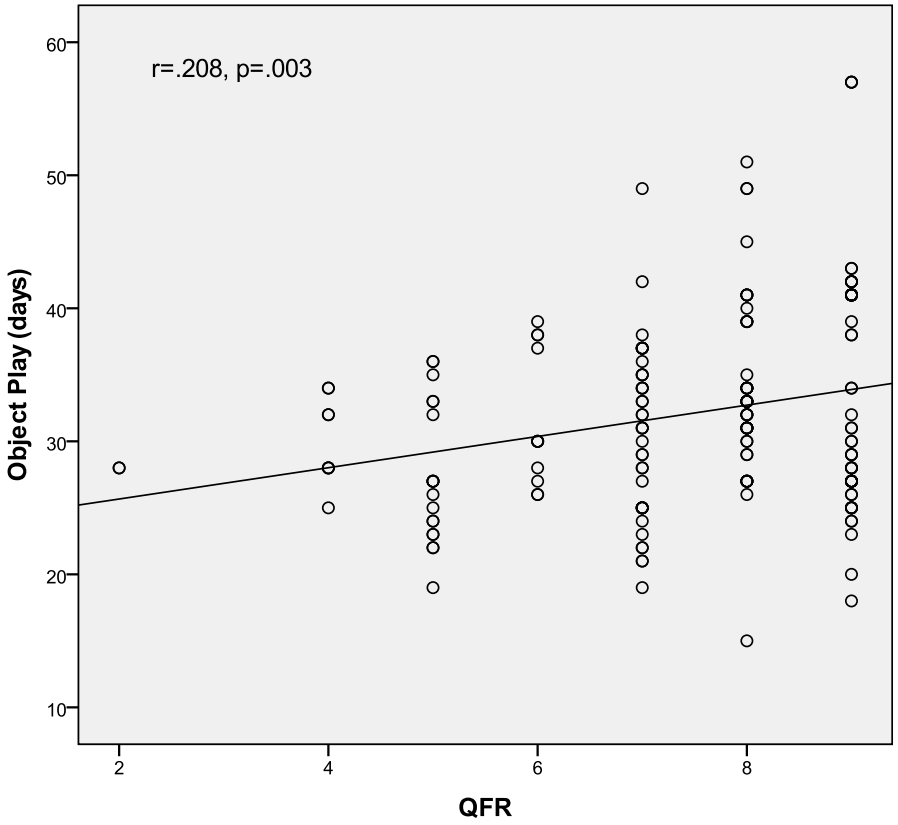


Figure 3.13. Scatter and line graph representing a positive relationship ($r^2 = 0.04$) between object play and queens' friendliness rating.

3.3.9. Litter Tray Usage

Tray usage was recorded as being the time at which the observer first noticed the kitten using the litter tray for elimination. Mean age for the population (n=212) as a whole was 32.98 days (*SE* 0.303).

No significant differences were found with regards to the timing of tray usage and percentage number of males ($r_s = -0.053$, $p = 0.446$), the kittens' breed (Kruskal-Wallis H2 = 1.279, p = 0.527), or age of the queen (Mann Whitney U = 2592.5, p = 0.630). Onset of tray usage was significantly associated with litter size. Kittens

derived from large litters (>4) used the tray at an earlier age (Mann Whitney U = 4475.5, p = 0.019).

QFR negatively correlated with timing of tray usage (r_s = -0.181, p = 0.011) (Figure 3.14), i.e. kittens from more friendliness queens used the litter tray at an earlier age. No significant correlation exists between timing of tray use and the kittens' birth weight (r_s = 0.056, p =. 0510).

Table 3.14 Descriptive statistics. Average onset of litter tray usage for young (≤24 months) and old queens (>24 months), small (≤4 individuals) and large litters (>4 individuals) and three main breed categories. Data includes number of cats for which data was reported, mean, SE and SD of number of days, median number of days since birth and IQR.

Tray		N	Age (days) Mean	Std. Error	Std Deviation	Median	IQR
Age of queen	Young mothers	134	32.92	0.346	4.008	32	6
	Older mothers	31	33.48	1.192	6.638	30	9
Litter Size	Small	97	33.91	0.518	5.097	33	8
	Large	68	31.76	0.416	3.430	30	6
Percentage males	≤33%	52	32.37	0.563	4.059	31	7
	34% to 66%	95	32.29	0.524	5.109	32	7
	>66%	18	33.50	0.673	2.854	34	5
Breed	Domestic	100	32.83	0.422	4.219	32	7
	Western	29	33.86	0.72	3.88	35	8
	Asian	36	32.89	0.999	5.994	31	5

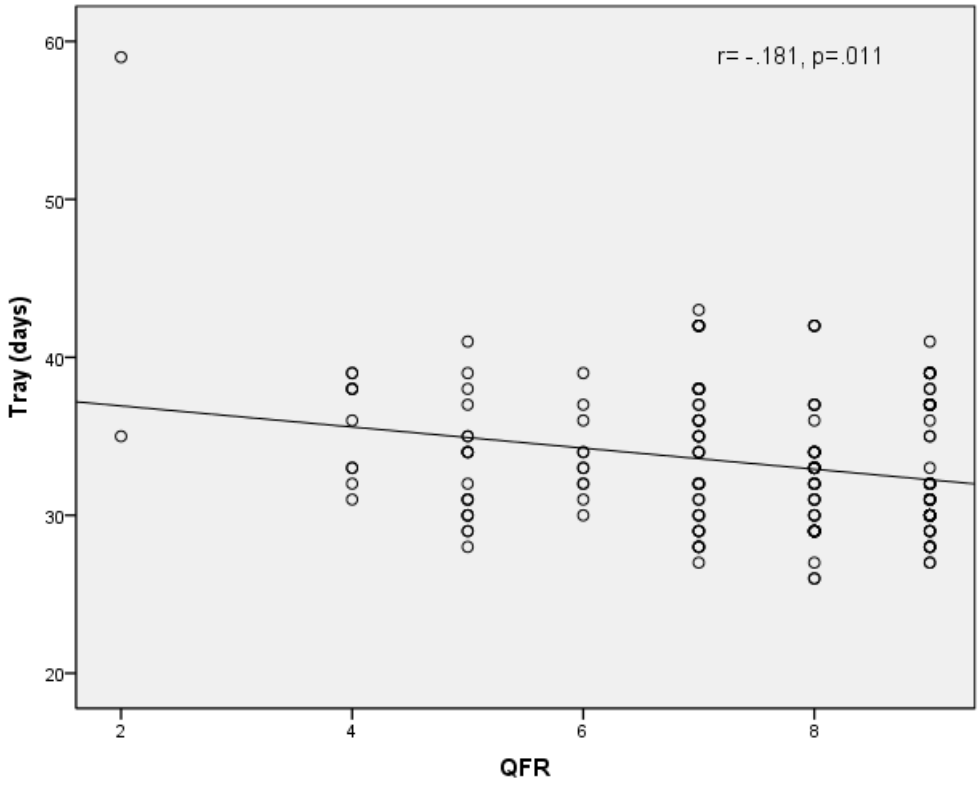


Figure 3.14. Scatter and line graph representing a negative relationship (r^2 = 0.032) between day when toileting started (days) and the queens' Friendliness Rating

Chapter 4. Discussion of findings and future research

The survey produced a picture of the complexity of effects of the nature of the queen and her litter on developmental milestones (Table 1.19, Figures 1.12-1.14). These findings can be summarised as Asian breeds produced larger litters with lower average birth weight than Western breeds or non-pedigree queens. Kittens from larger litters (5 or more kittens) opened their eyes earlier than smaller litters, however had a generally delayed onset of other subsequent developmental milestones with the exception of use of the litter tray. Kittens from older queens (over 2 years of age) showed earlier onsets of eye opening, but later tooth eruption, play and movement from nest than kittens derived from younger queens.

Queens rated as being unfriendly to humans produced smaller litters with a female bias. Litters from friendly queens also showed earlier tooth eruption, solid feeding and litter tray use but delayed object play compared to litters from less friendly queens. The picture is however complex, with advanced early milestones (such as eye opening) often associated with delayed later milestones such as locomotion and play and the effects of each factor will be discussed in turn.

4.1. Litter size

Birth weight was inversely correlated with litter size, which concurs with previous research (e.g. Gatel et al, 2011; Hudson, 2011; Rodel, 2008), and as we were studying natural or un-manipulated litters, the impact of these two factors are difficult to untangle. Our results show that individuals derived from larger litters

opened their eyes earlier, whereas individuals from small litters exhibited earlier onset of play and mobility.

Litter size has been documented to be a factor in the onset of development in a number of species (Guerra et al, 2001; Rodel, 2010). Small litters of Long Evan laboratory rats have a later onset of development, specifically motor ability directed at reaching central litter positions, compared to large litters (Bautista et al, 2010). Species dependent PNS has also been implicated in both lowering and increasing birth weight depending upon the type of stressor inflicted (Roussel *et al*, 2004, Mueller & Bale, 2006; Emack *et al*, 2008; Xiaobai *et al*, 2014). Inverse correlations were found between birth weight and eye opening, movement of kittens from the nest and self play behaviour, thus indicating those individuals with higher birth weight develop at a quicker rate. Large litters may have a slower development due to increased competition for resources (Bautista et al, 2010) or may reach milestones sooner as the litter size is a reflection of resources available to the queen. This effect could be studied in more detail under controlled conditions, for example by cross fostering between litters to artificially manipulate birth weight and litter size.

4.2. Age of the queen at parturition

Our study investigated the potential influence of queen's age on development, including litter variables. Younger queens (≤24months) produced kittens with significantly earlier onsets in a number of later developmental traits, such as tooth eruption, food consumption, moving from the nesting area and sibling play. However, older queens had an earlier onset of the first developmental onset as their kittens opened their eyes at an earlier age. Though eye opening occurred later in young queens, the actual speed of development appeared to increase in this group

over time. It has been found in a range of species including man (e.g. Mercer and Ferketich 1995) and rodents (Bridges et al 1985) that inexperienced mothers may show limited care giving, or increased levels of prenatal stress, increasing circulatory glucocorticoid reaching the placental, thus affecting the number of stress receptors within offspring (Welberg et al 2001, Weinstock 2008). As a consequence, it could be hypothesised that independence from the mother such as self sufficiency in food ingestion and enhanced motor abilities develop at a quicker rate, though this would be dependent on the severity of the stressor (Braastad, 1998). As well as experience of care of offspring, a number of other maternal factors may also have varied with age. These include the queen's cumulative experience of environmental stressors, which may influence her stress physiology during gestation, as well as her nutritional state and body condition. Further work planned as part of this study, would have included data on queen's nutrition and body condition, as well as independent measures of stress physiology and temperament.

Offspring derived from rat dams subjected to PNS in the last week of gestation have been shown to have faster behavioural and physiological development though no change in time of eye opening by rat pups (Fride & Weinstock, 1984). In our study, a high proportion of younger queens were derived from a shelter population. In many instances, such individuals were relinquished in the later stages of gestation, and many were fostered in their last week of gestation (queens received ultrasound tests to assess if their gestation was post 5 weeks, if earlier queens were neutered by rescue charity). Depending on the individual, PNS may have been encountered on arrival to the shelter, during their stay (blood testing, ultrasound, housing etc) and/or when moved to a foster home. This may reflect the findings of Fride & Weinstock (1984) and is an area where further research is required, for

example measuring physiological and behavioural measures of PNS. In this study, there was no relationship between the age of the queen and birth weight of offspring, nor litter size which concurs with other studies (Gatel et al, 2011). However, there was a significant effect of the age of the queen on the percentage number of males within the litter; the number of males increased with the age of the queen.

Trivers and Willard (1973) suggest that mothers in good condition will produce the sex with the highest fitness and greatest investment when taking into consideration environmental stressors. To the best of our knowledge, this effect has not previously been reported in felid species, and whilst the evidence in this study is noteworthy, it must also be noted that our study had not set out to investigate the Trivers-Willard effect, and corroborating evidence from other studies, where queen's condition during pregnancy was systematically monitored, would be necessary before firm conclusions could be drawn. Nevertheless, if our findings were confirmed, then it could be argued that our findings are consistent with an increase in males in low stress environment, and females in high stress induced environments, if maternal post copulatory selection is possible in domestic cats.

In rodents, male pups appear to be more affected by PNS than females (Bhatnagar et al, 2005; Emack et al, 2008). As such, for an individual to produce a high male ratio into a stressful environment may not be the optimal evolutionary strategy, as males may have less chance of survival. Emack et al (2008) found that male guinea pigs born to PNS mothers exhibit lower growth rates and reduced open field ambulatory activity, which did not occur in females. Smaller female biased litters may have been produced as result of PNS in our study, (omitting breed effects and maternal condition), which concurs with Helle *et al*, (2008) whose research into voles propose that larger litters (and older females) produced a high male bias within

litters, and would therefore be in agreement with the Trivers and Willard hypothesis (1973).

Our study showed that male dominated litters move from the nest and engaged in self-play behaviours at an earlier age. As this data was derived from carer observation alone, it may be worthy to analyse the effect of sex bias in a more controlled setting and focusing on specific motor abilities than generalised behavioural patterns, whilst taking into account of the actual sex of the kitten, in addition to the sex ratio.

4.3. Breed type

Kittens from Asian queens had lower birth weight than the two other groups and their litter sizes were significantly larger. Litter sizes and birth weights are likely be due to genetic factors, which are reflected in other studies that have directly looked at behavioural and physiological difference due to breed types in kittens (Marchei *et al*, 2009; Marchei *et al*, 2011; Gatel *et al*, 2011). These studies concur with the physiological and behavioural onsets witnessed in our study, such as earlier onset of eyes opening in Asian kittens compared to domestic and Western breeds. This is suggestive that Asian breeds initial neurological development is quicker which concurs with Marchei et al (2009), however, the sex ratio shift towards a female bias is unclear and is worthy of wider attempts at replication.

Play behaviours, both object and self play had a later onset in Asian breeds; which may be due to breed specific developmental onsets (see table 1.19). Marchei et al (2009) tested different breeds in an open field test. They found that Asian related breeds, such as Oriental, Siamese and Abyssinian were more reserved when approaching a novel object compared to breeds such as the Norwegian Forrest. Marchei et al (2009) related this alteration in behaviour to individual breed's coping

style. They suggest that Asian breeds are “shy” compared to a “bold” style exhibited by the Norwegian forest. They also suggest that the capability of an earlier onset in neurological and emotional development in Asian breed type cats predisposed them to earlier fear-related memory and subsequent learning of fear-induced stimuli alongside related stress-induced physiological functions. Therefore, they were able to learn that a novel object did not pose a threat when re-exposed. They also note that these two groups show altered arousal levels to stimuli; Asian breeds required higher arousal evoking stimuli, therefore have been suggested to have a higher arousal threshold (Marchei et al, 2011). When subjected to a novel object, Norwegian forest kittens spent a greater time exhibiting stress arousing behaviours such as facial rubbing a novel object than Asian breeds. However, after a threatening stimulus was introduced into the environment, Asian breeds were witnessed exhibiting higher exploratory and rubbing of the original novel object.

A similar comparative study to assess the emotional abilities and associated levels of maturation of domestic breed kittens, to include individual differences depending upon potential factors such as timing of developmental onsets should prove to be an important method of determining potential coping styles and arousal levels in adulthood. Potential factors may include the health status of the queen, body weight, BMI or fat levels, the litter size and sex ratio, individual positioning within the little (huddle positioning). The latter has been documented to influence individuality within litters (Bautista et al 2010) and has yet to be thoroughly studied within the cat.

Overall our results are suggestive that a range of maternal factors contribute towards timing of developmental onsets, more specifically they show evidence of an

association between increased maternal stress response during the prenatal period, litter variables and developmental onsets in cats.

4.4. Queen's Friendliness Rating

A carer derived assessment of personality found that there was a small positive relationship between QFR and the number of males within the litter, e.g. the friendlier the queen then more males she produced. This finding is similar to Bakken (1997) whose study of sociability of farmed silver fox and subsequent litter sex ratios found that vixens provided with a positive reinforcer twice weekly during gestation (a titbit which had been previously tested as a reliable method to reduce the foxes fear of human), were more likely to produce litters with a high male sex bias compared to the control group. In addition, queens classed as unfriendly in our study only produced small litters, whereas friendly queens produced both small and large litters.

It has been stated that increased litter size is generally associated with lower individual body masses and that these individuals may also show retarded growth (Tanaka, 1998), however retarded growth has also been found to be as a result of mild maternal chronic stress (Xiaobai et al, 2014). In the latter study, there is some debate as to the definition of "mild stress", stressors in this study consisted of multiple types, including strobe lighting at unpredictable times. Whereas even milder stressors that have not manifest as anxiety in adult offspring during the prenatal period have been found to influence offspring (Mabandla et al, 2008), where mild stress provokes better coping abilities (Catalani et al, 2011). In our study, developmental timing may be delayed in large litters, as larger litters have been documented to be produced in a less stress-induced environment; such

individuals have the time and recourses to “catch up”. In essence, they do not need to develop at a quick rate for their survival. Competiveness between individuals within larger litters may induce stress, but at a low level and may, in fact, help to promote reduced stress reactivity in adulthood. Rodel et al (2010) found that heavier Long-Evan laboratory rats from smaller litters had higher corticosteroid levels than lighter individuals from large litters when tested at post-natal day 17. They concluded that the challenges of constant competition and reduced maternal resources were a repeated mild stressor in the larger litters.

QFR showed an inverse correlation with tooth eruption, eating solid food, and tray usage. These results may be due to the observers’ ability to correctly witness and record behaviours, i.e. friendlier queens will allow handling of their offspring therefore tooth eruption may have been witnessed and recorded more easily. Alternatively, kittens who witness their mother interact and use her environment in the presence of an observer will themselves be encourage to display similar behaviours (Bradshaw et al, 2012). This does not mean that kittens from unfriendly queens do not have the capacity at a physiological level to carry out such behaviours. Alternative recording methods could be utilised to record behavioural milestones, e.g. video recording utilising small cameras rigged in and around the nesting area. Whatever the cause for the delay in such behaviour, further long-term study of the effects of housing unfriendly queens, such as PNS and their subsequent effects on behavioural development of kittens is required. Such results may also help develop recommendations for changes to husbandry practises. In addition, the effects of QFR may also prove to be a factor in determining kittens’ arousal levels and coping styles.

4.5. PNS and Animal Welfare

It could be advantageous to suggest that PNS may have serious implications on animal welfare, dependent on the level of subjective PNS. A range of interdependent, species specific factors would need to be categorised, in order for PNS and its influences on individual difference to be truly understood. However, as current research models are constructed to evaluate the effect and predictability of PNS on human emotional and cognitive dysfunction and are recognised as a contributing factor in the formation of many human psychological disorders, this research could cross over into veterinary behavioural medicine.

Such research would suggest that other domesticated animals or those held in captivity, including kittens derived from Queens experiencing PNS during late gestation, such as under-socialised queens, or those with lowered stress arousal, may also exhibit similar patterns of behaviours; such as heightened stress responses, lowered stress thresholds, reduced cognitive functions. However, it may by hypothesised that feline welfare implications could manifest as an increased tendency of anxiogenic expressions that may include aggression and heightened fear responses.

4.6. Methodological Discussions, Conclusions and Further research.

The findings of these studies indicate that developmental milestones are not chronologically fixed in domestic cats and variation in onsets is related to a number of queen and litter factors, which require further investigation. The study found breed and litter size effects on developmental milestones were broadly consistent with previous research in cat development, and suggested novel factors such as

queen's temperament and sex ratios of litters may also affect these developmental milestones. It should be noted that the data was entirely derived from carer survey, and although the two sub-populations that made up the sample, queens in rescue shelters and breeding queens are likely to have carers with both the knowledge and motivation to maintain good records, the data collection was dependent on the carer's ability and opportunity to maintain accurate records. As a consequence, some data was missing due to carers not being able to detect all onsets, and there will be some variation in data set related to carers. Nevertheless, a number of clear patterns emerged relating to easily identifiable onsets that are unlikely to have been influenced by carer attributes. These include the earlier onset of eye opening by kittens from larger litters and older queens, but subsequent delayed onsets attributed to these factors.

A second consequence of the survey approach was the makeup of the cat population surveyed, being made up of largely pedigree breeds under the care of cat breeders and a contrasting population of non-pedigree cats and kittens in a rescue, rehabilitation and rehoming centre, largely derived from feral, stray or abandoned cats. Although these two populations only represent a subset of breeding population on a global scale (and do not include casual breeding in pet owning population, or free-living feral populations), they form a representative sample of breeding cats in the UK, where there have been long-standing campaigns to neuter cats in both the owned and feral/stray populations to control cat populations (Murray et al 2015). As well as breed differences, there was a clear distinction in temperament from these sub-populations, with the queens in the pedigree population being rated as generally friendly, whilst rescue queens including both friendly and unfriendly individuals. It is therefore difficult to directly compare the effect of friendliness in these two

populations, partly as only one pedigree cat was described as unfriendly and partly, even when categories are clearly defined the carers may have preconceived expectations of the cat's behaviour that may bias their assessment of temperament. Future work should therefore include some validation of the temperament assessment and the accurate detection of developmental onsets, for example including an independent direct assessment, supported by video evidence in form of video diaries. As part of this approach it would also be beneficial to assess if the initial QFS used in the pilot survey is fir for purpose, or if multiple scales should be used to distinguish between aggressiveness and nervousness in "unfriendly" cats.

Although this survey introduced an assessment of queen's temperament which may relate to queen's perception of human intervention and potentially their response to prenatal stressors, further data should be gathered to test this relationship and control for other prenatal environmental factors. Development of the scale would include developing the tool to assess both socialisation and boldness of the Queen. Assessment could then partly be validated by the use of video records and independent assessment as mentioned already, but also by collecting data on the queen's physiological state during gestation. This could for example include assay of corticosteroids from urine to assess medium term activation of HPA, and collection of saliva or mucus to measure Immunoglobulin levels (IgA). In addition, queen's condition can be monitored by body score measurements and regular weighing as well as monitoring feed intake to assess nutritional intake. Whilst we would anticipate that cat breeders and rescue shelters would ensure cats in their care have good nutrition and health intervention, an initial assessment of condition in the early stages of gestation/care would be useful, particularly in the rescue population.

These additional controls were to be included in studies that would have extended the study to a PhD as originally envisaged.

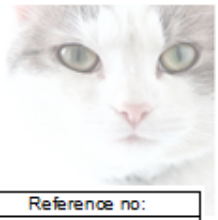
Finally, future work is needed before exact feline husbandry recommendation can be formalised and would require learning if differences in behaviour, stress reactivity and its impact on health in adulthood can be linked to maternal care and early experiences, including socialisation of kittens during ontology. However, it can be hypothesised form this and other research that maternal care and stress reactivity during gestation and after birth can have a significant effect on the development of offspring. General husbandry guidance would therefore include minimalizing prenatal stress by reducing potential environmental stressors, ensuring optimum environmental resources are always available, avoid forced interactions between caregivers and the queen, and allowing the queen a sense of choice over her interactions and environmental preferences throughout gestation and after parturition.

Appendix A

Kitten Development Record

UNIVERSITY OF LINCOLN

WOOD GREEN The Animals Charity



We are trying to gather important information to improve our knowledge and understanding of feline development, particularly kitten development. We would like you to provide us with some basic information about your pregnant queen and the stud (where available), in addition to some more in depth information relating to the subsequent litter of kittens. We particularly need the date of the kitten's birth and your observations of the kittens as they grow.
When filling out the behaviour chart please record the date on which you **first** notice the behaviours/physical attributes which are listed. When you have completed the form, please remember to fill in your details and then send it back in the pre-paid envelope provided! If you have any questions please do not hesitate to call or email, details are at the bottom of this form.

Your details	Reference no:
Name:	
Address:	
Post Code:	
Telephone number:	
Email:	
Preferred method of contact:	Email / Phone / Address

Cats details

Queen

Name:	Age of Queen when she gives birth: months
Breed:	
Coat colour and length:	
Any known health problems (e.g. heart murmur):	
Any known illnesses during pregnancy/lactation. e.g. mastitis, cat flu etc:	

Please indicate on the scale below the temperament of your queen towards people (1 being very aggressive and 9 very friendly)

(very aggressive) 1 2 3 (shy) 4 (ignores) 5 (approaches) 6 7 8 9 (Very friendly)

Tom

Name:	Age:
Breed:	
Coat colour and length:	
Any known health problems:	

Please indicate on the scale below the temperament of your tom cat towards people (1 being very aggressive and 9 very friendly)

(very aggressive) 1 2 3 (Shy) 4 (ignores) 5 (approaches) 6 7 8 9 (Very friendly)

Kittens details

Kittens date of birth: (dd/mm/yyyy)

	Name	Sex	Coat colour	Coat length	Breed / Type
Kitten 1					
Kitten 2					
Kitten 3					
Kitten 4					
Kitten 5					
Kitten 6					
Kitten 7					
Kitten 8					

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Work: 0844 248 8181 ext 1305

Kitten Development Record

There are spaces at the top of each chart to write the name of each kitten which should correspond to the kitten details chart on the previous page. Please record weight below in **GRAMS**.

Please record kitten date and weight (IN GRAMS) weekly	Date	Kitten's name							
		1	2	3	4	5	6	7	8
	Weight at birth **Date:**								
	week 1 **Date:**								
	week 2 **Date:**								
	week 3 **Date:**								
	week 4 **Date:**								
	week 5 **Date:**								
	week 6 **Date:**								
	week 7 **Date:**								
	week 8 **Date:**								
	week 9 **Date:**								
	week 10 **Date:**								
	week 11 **Date:**								
	week 12 **Date:**								

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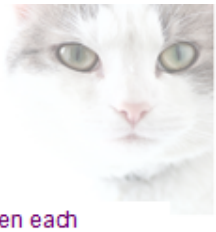
Kitten Development Record

UNIVERSITY OF LINCOLN

WOOD GREEN The Animals Charity



Please complete each section where possible. Please write in the boxes the **date** when each behaviour or physical attribute was first noticed for **each kitten** in the litter. If an attribute is not seen please enter **NS** in the box instead of the date. For an explanation of the attribute/behaviour please refer to the sheet at the back of this handout.

Please note the date when the first instance is seen.

Behaviour/ Physical attribute	Kittens name							
	1	2	3	4	5	6	7	8
Eyes start to open								
Eyes are fully open								
Queen moving kitten out of the bedding area								
Kitten moving itself out of the bedding area								
Teeth start to erupt								
Eating solid food								
Using the litter tray								
Self-play – play on its own								
Play - with litter mates								
Play – with object								
Purring								
Scratching e.g. on carpet / scratch post								

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Kitten Development Record

Do you allow them to mix with your queen and kittens? YES/NO

Notes and definitions

Eyes start to open: The point at which you first notice the opening of the lids of one or both eyes, you will normally see a small slit where the eye can partially be seen.

Eyes completely open: Both eyes are completely open, this takes approximately one to two days after the eyes initially start to open, but may take longer.

Queen moving kitten out of the bed: This may or may not happen. In addition it may only occur to some individuals of the litter and not others. Sometimes the queen will pick up the kitten by the scruff of the neck and move it outside of the nest/bed.

Kitten moving itself out of the bedding area: The kittens will start to move around more as they develop. With time they will start to venture from the bedding area to explore.

Teeth start to erupt: When the first tooth is visible.

Eating solid food: The first instance you notice that the kitten is eating food provided by yourself.

Using the litter tray: You may find it difficult to distinguish between individuals using the tray as you may not be around to witness it, or you may have a large litter of kittens.
If you are able to determine who is using the tray then please just write the date down for that individual in the relevant box. However, if it is difficult, please put a line through the whole row and just write down the date of the first time you noticed that a kitten has used the tray above this line.

Self play – play on its own: The kitten plays on its own – no interaction with littermates.

Play with litter mates – social play: Play between individuals.

Play with objects: Play that involves the kitten using a toy to play with on its own accord, such as batting a ball.

Purring: When the kitten vocalises and produces a purring noise.

Scratching – posts, carpet, objects: The kitten will grip an object with both front limbs, extends it claws and scratches.

Thank you for donating your time in completing this form!

Once complete just send this form back in the pre-paid envelope provided.
If you have any comment regarding this form, please fill in the space below. Thank you.

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References

Bakken, M. (1997) The effect of an improved man-animal relationship on sex ratio in litters and on growth and behaviour in cubs among farmed silver fox (*Vulpes vulpe*s). *Applied Animal Behaviour Science*. **56**: 309-317.

Bakker, J. M., Frank van Bel. & Heijnen, C. J. (2001) Neonatal glucocorticoids and the developing brain: short-term treatment with life-long consequences? *Trends in Neuroscience*. **24**: 649-653.

Barbazanges, A., Piazza, P. V., Le Moal, M. & Maccari, S. (1996) Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *Journal of Neuroscience*. **16**: 3943-3949.

Bateson, P. (1979) How do sensitive periods arise and what are they for? *Animal Behaviour.* **27**: 470-486.

Bateson, P. (2000) Behavioural development in the domestic cat. In: *The domestic cat. The biology of its behaviour (2nd Ed)*, ed. Turner, D. C. & Bateson, P, pp. 10-22. Cambridge: Cambridge University Press.

Bautista, A. (2010) Development of behaviour in the litter huddle in rat pups: Within- and between- litter differences. *Developmental Psychobiology*. **52**: 35-43.

Beaver, B. (2003) Feline behaviour. A guide for veterinarians. St Louis, Missouri: Saunders.

Bhatnagar, S., Lee, T. M. & Vining, C. (2005) Prenatal stress differentially affects habituation of corticosterone response to repeated stress in adult male and female rats. *Hormones and Behavior.* **47**: 430-438.

Bowman, R. E., MacLusky, N.J., Sarmiento, Y., Frankfurt, M., Gordon, M. & Luine V. N. (2004) Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. *Endocrinology.* **145:** 3778-87.

Boersma, G. J. & Tamashiro, K. L. (2015) Individual differences in the effects of prenatal stress exposure in rodents. *Neurobiology of Stress*. **1**: 100-108

Braastad, B. O. & Heggelund, P (1984) Eye-opening in kittens: effects of light and some biological factors. *Developmental Psychobiology*. **17**: 675-81

Braastad, B. O. (1998) Effects of prenatal stress on behaviour of laboratory and farmed animals. *Applied Animal Behaviour Science.* **61**: 159-180.

Bradshaw, J. W. S. (1992) The behaviour of the domestic cat (1st ed.). Wallingford: CABI Publishing.

Bradshaw, J. W. S., Neville, P, F and Sawyer, D (1997) Factors affecting pica in the domestic cat. *Applied Animal Behaviour Science*. **52**: 373-379.

Bradshaw J. W. S., Casey R. A. & Brown S. L. (2012) The Behaviour of the Domestic Cat (2nd ed.). Wallingford: CABI Publishing.

Bremner, J. D., Vythilingam, M., Vermetten, E., Southwick, S. M., McGlashan, T., Nazeer, A., Khan, S., Vaccarino, L. V., Soufer, R., Garg, P. K., Ng, C. K., Staib, L. H., Duncan, J. S. & Charney, D. S. (2003) MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and post-traumatic stress disorder. *American Journal of Psychiatry*. **160**: 924-932.

Bridges, R. S., DiBiase, R., Loundes, D. D. & Doherty, P.C. (1985) Prolactin stimulation of maternal behavior in female rats. *Science*, **227**: 782-784.

Butkevich, I., Mikhailenko, V. A., Vershinina, E. A., Semionov, P. O., Otellin, V. A. & Aloisi, A. M. (2009) Heterogeneity of the infant stage of rat development: Inflammatory pain response, depression-related behaviour and effects of prenatal stress. *Brain Research*. **1286**: 53-59.

Caldji, C., Diorio, J. & Meaney, M.J. (2000) Variations in Maternal Care in Infancy Regulate the Development of Stress Reactivity. *Biological Psychiatry*.**48**:1164–1174

Cameron, E. Z. (2004) Facultative adjustment of mammalian sex ratios in support of the Trivers–Willard hypothesis: evidence for a mechanism. *Proceedings of the Royal Society B: Biological Sciences*. **271**: 1723-1728

Campion, J., Milagro, F.I. & Martinez, J.A., 2009. Individuality and epigenetics in obesity. *Obesity Reviews*. **10**: 383-392.

Casey, R. A. & Bradshaw, J. W. S. (2007) The assessment of welfare. In: *The Welfare of Cats*, ed. Rochlitz, I, pp.23-46. Dordrecht, The Netherlands: Springer.

Casey, R. A. & Bradshaw, J. W. S. (2008) The effects of additional socialisation for kittens in a rescue centre on their behaviour and suitability as a pet. *Applied Animal Behaviour Science.* **114:** 196–205

Catalani, A., Alema, G. S., Clinque, C., Zuena, A. R. & Casolini, P. (2011) Maternal corticosterone effects on hypothalamus-pituitary-adrenal axis regulation and behaviour of the offspring in rodents. *Neuroscience and Bio-behavioural Reviews*. **35**: 1502-1517.

Champagne, F. A. & Curley, J. P. (2009) Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neuroscience and Bio-behavioural Reviews.* **33**: 593-600.

Chen, Q., Zhang, Y., Peng, H., Lei, L., Kuang, H., Zhang, L., Ning, L., Cao, Y. & Duan, E. (2011) Transient beta 2-adrenoceptor activation confers pregnancy loss by disrupting embryo spacing at implantation. *Journal of Biological Chemistry*. **286**:4349-56

Charil, A. Laplante, D. P., Vaillancourt, C. & King, S. (2010) Prenatal stress and brain development. *Brain Research News.* **65:** 56-79.

Clarke, A. S., Soto, A., Bergholz, T. & Scheider, M. L. (1996) Maternal gestational stress alters social behaviour in adolescent rhesus monkey offspring. *Infant Behaviour and Development.* **19**: 451-461.

Claus, M. A., Levy, J. K., MacDonald, K., Tucker, S. J & Crawford, P. C. (2006) Immunoglobulin concentrations in feline colostrum and milk, and the requirement of colostrum for passive transfer of immunity to neonatal kittens. *Journal of Feline Medicine and Surgery.* **8**: 184-191.

Couret, D., Prunier, A., Mounieer, A., Thomas, F., Oswald, I. P. & Merlot, E. (2009) Comparative effects of a prenatal stress occurring during early or late gestation on pig immune response. *Physiology and Behaviour.* **98**: 498-504.

Darlington, R. B., Dunlop, S. A. & Finlay, B. L. (1999) Neural development in metatherian and eutherian mammals: Variation and constraints. *The Journal of Comparative Neurology*. **411**: 359-368.

Deag, J. M., Manning, A. & Lawrence, C. E. (1987) The consequences of differences in litter size for the nursing of cat and kittens. *Journal of Zoology.* **213**: 153-179.

Deag, J. M., Manning, A. & Lawrence, C. E. (2000) Factors influencing the mother-kitten relationship. In: *The domestic cat. The biology of its behaviour (2nd Ed)*, ed. Turner, D. C. & Bateson, P, pp. 24-45. Cambridge: Cambridge University Press

Del Cerro, M, C, R., Perez – Laso, C., Ortega, E., Martin, J. L. R., Gomez, F., Perez-Lzquierdo, M. A. and Segovia, S. (2010) Maternal care counteracts behavioural effect of prenatal environmental stress in female rats. *Behavioural Brain Research*. **208**: 593-602.

D'Eath, R.B., 2005. Socialising piglets before weaning improves social hierarchy formation when pigs are mixed postweaning. *Applied Animal Behaviour Science*. **93**: 199–211.

Emack, J., Kostaki, A., Walker, C. D., Matthews, S. G. (2008) Chronic maternal stress affects growth, behaviour and hypothalamo-pituitary-adrenal function in juvenile offspring. *Hormones and Behaviour.* **54**: 514-520.

Estanislau, C. & Morato, S. (2006) Behaviour ontogeny in the elevated plus-maze: prenatal stress effects. *International Journal of Developmental Neuroscience*. **24**: 255-262.

Finka, L. R. (2015) The prediction of Human-sociability in the domestic cat *Felis silverstris catus.* PhD. University of Lincoln.

Fleming, A.S., Kraemer, G.W., Gonzalez, A., Lovic, V., Rees, S., Melo, A. (2002) Mothering begets mothering: the transmission of behavior and its neurobiology across generations. *Pharmacology, Biochemistry and Behaviour*. **73**: 61–75.

Foyer, P., Wilson, E. & Jensen, P. (2016) Levels of maternal care in dogs affect offspring temperament. *Scientific Reports*. 6: 19253.

Fride, E. & Weinstock, M. (1984) The effects of prenatal exposure to predictable or unpredictable stress on early development in the rat. *Developmental Psychobiology.* **17**: 651-660.

Gartner, M. C. & Weiss, A. (2013) Personality in felids: A review. *Applied Animal Behaviour Science*. **144**: 1-13.

Gatel, L., Rosset, E., Chalvet-Monfray, K., Buff, S. & Rault, D. N. (2011) Relationships between foetal biometry, maternal factors and birth weight of purebred domestic cat kittens. *Theriogenology*. **76**: 1716-22

Green, M, K., Rani, C.S.S., Joshi, A., Soto-Piña, A.E., Martinez, P.A., Frazer, A., Strong, R. & Morilak, D.A. (2011) Prenatal stress induces long term stress vulnerability, compromising stress response systems in the brain and impairing extinction of conditioned fear after adult stress. N*euroscience.* **192**: 438–451

Gonzalez, A. & Fleming, A.S. (2002). Artificial rearing causes changes in maternal behaviour and c-fos expression in juvenile female rats. *Behavioural. Neuroscience*. **116**. 999–1013.

Gonzalez, A., Lovic, V., Ward, G.R., Wainwright, P.E., Fleming, A.S. (2001) Intergenerational effects of complete maternal deprivation and replacement stimulation on maternal behaviour and emotionality in female rats. *Developmental Psychobiology*. **38**:11–32.

Gotz, A. A., Wolf., M. & Stefanski, V. (2008) Psychosocial maternal stress during pregnancy: Effects on reproduction for F0 and F1 generation laboratory rats. *Physiology and Behaviour*. **93**: 1055-1060.

Guerra, R. F. & Nunes, C. R. de O. (2001) Effects of litter size on maternal care, body weight and infant development in golden hamsters. *Behavioural Processes*. **55**: 127-142.

Guhad, F.A., Hau, J. (1996) Salivary IgA as a marker of social stress in rats. *Neuroscience Letters*. **216**: 13-140.

Guyot, G.W., Cross, H. A. & Bennett, T. L. (1977) Early socialisation of the domestic cat: Responses to separation from social and non social rearing stimuli. *Developmental Psychobiology*: **13:**309-315

Hart, B. L (1976) Behavioural aspects of selecting a new cat. *Feline Practice*. **6**: 8, 10, 14

Harley, R., Gruffydd-Jones, T. J & Day, T, J. (1998) Determination of salivary and serum immunoglobulin concentrations in the cat. *Veterinary Immunology and Immunopathology*. **65**: 99-112.

Harlow, H. F., Dodsworth, R. O. & Harlow, M, K. (1965) Total social isolation in monkeys. *Proceedings of the National Academy of Science of the United States of America*. **54**: 90-97.

Harlow, H.F.& Suomi, S.J. (1971) Social recovery by isolation-reared monkeys. *Proceedings of the National Academy of Science of the United States of America*. **68**: 1534–1538.

Helle, S., Laaksonen, T., Adamsson, A., Paranko, J. & Huitu, O. (2008) Female field voles with high testosterone and glucose levels produce male-biased litters. *Animal Behaviour*. **75**: 1031-1039

Hemsworth, P. H. & Barnett, J. L. (2003) The effects of early contact with humans on the subsequent level of fear of humans in pigs. *Applied Animal Behaviour Science.* **35:** 83-90.

Henry, C., Kabbaj, M., Simon, H., Le Moal, M and Maccari, S. (2004) Prenatal Stress Increases the Hypothalamo-Pituitary-Adrenal Axis Response in Young and Adult Rats. *Journal of Neuroendocrinology.* **6:** 341-345.

Hougaard, K., Andersen, M., Kjaer, S., & Hansen, Å. M., Werge, T & Lund, S. (2005). Prenatal stress may increase vulnerability to life events: Comparison with the effects of prenatal dexamethasone. *Brain Research*. **159**. 55-63.

Hudson, R., Bautista, A., Reyes-Meza, V., Montor, J. M. & Rodel, H. G. (2011) The effect of siblings on early development: A potential contributor to personality differences in mammals. *Developmental Psychobiology*. **53**: 564-574.

Jarvis S., Moinard C., Robson S.K., Baxter E., Ormandy E., Douglas A.J., Seckl J.R., Russell J.A & Lawrence A.B. (2006) Programming the offspring of the pig by prenatal social stress: Neuroendocrine activity and behaviour. *Hormones and Behavior*. **49:** 68-80.

Jensen, R. A., Davis, J. L. & Shnerson, A. (1980) Early experience facilitates the development of temperature regulation in the cat. *Developmental Psychobiology*. **13:** 1-6.

Jones, K, L., Smith, R. M., Edwards, K. S., Givens, B., Tiley, M. R. & Beversdorf, D. Q. (2010) Combined effect of maternal serotonin transporter genotype and prenatal stress in modulating offspring social interaction in mice. *International Journal of Developmental Neuroscience.* **28**: 539-536.

Kapoor, A. & Matthews, S. (2005) Short periods of prenatal stress affect growth, behaviour and hypothalamo–pituitary–adrenal axis activity in male guinea pig offspring. *Journal of Physiology.* **556:** 967-977.

Kapoor, A., Dunn, E., Kostaki, A., Andrews, M. H. & Matthews, S. G. (2006) Fetal programming of hypothalmo-pituitary-adrenal function: prenatal stress and glucocorticoids. *Journal of Physiology*. **572:** 31-44.

Kapoor, A., Petropoulos, S. & Matthews, S. G. (2008) Fetal programming of hypothalamic -pituitary–adrenal (HPA) axis function and behavior by synthetic glucocorticoids. *Brain Research Reviews.* **57**: 586-595.

Kash, E. B. & Turner, D. C. (1988) The human-cat relationship. In: The Domestic Cat: the biology of its behaviour, (1st ed.), pp 159-177. Ed D. C. Turner & P. P. G. Bateson. Cambridge: Cambridge University Press.

Kikusui, T & Mori, Y. (2003) Early weaning induces anxiety and aggression in adult mice. *Physiology & Behaviour* **81**: 37– 42.

Kikusui, T., Takeuchi, Y. & Mori, Y. (2005) Early weaning deprives mouse pups of maternal care and decreases their maternal behaviour in adulthood. *Behavioural Brain Research.* **162**: 200–206.

Kikusui, T., Nakamura, K. & Mori, Y. (2007) A review of the behavioral and neurochemical consequences of early weaning in rodents. *Applied Animal Behaviour Science.* **110**: 73–83.

Kikusui, T & Mori, Y. (2009) Behavioural and neurochemical consequences of early weaning in rodents. *Journal of Neuroendocrinology***. 21**: 427-431.

Kinsley, C. & Svare, B. (1987) Genotype modulates prenatal stress effects on aggression in male and female mice. *Behavioural and Neural Biology.* **47**: 138-150.

Koehl, M., Darnaudéry, M., Dulluc, J., Van Reeth, O., Le Moal., M. & Maccari, S. (1999) Prenatal stress alters circadian activity of hypothalamo–pituitary–adrenal axis and hippocampal corticosteroid receptors in adult rats of both gender. *Developmental Neurobiology*. **40:** 302-315.

Kranendonk, G., Hopster, H., Fillerup, M., Dinand Ekkel, E., Mulder, E. J. H., Wiegant, V. M. & Taverne, M. A. M. (2006) Lower birth weight and attenuated adrenocortical response to ACTH in offspring from sows that orally received cortisol during gestation. *Domestic Animal Endocrinology.* **30**: 218–238

Krohn, C. C., Jago, J. G. & Boivin, X. (2001) The effect of early handling on the socialisation of young calves to humans. *Applied Animal Behaviour Science*. **74:** 121-133.

Lemaire, V., Koehl, M., Le Moal, M & Abrous, D. N. (2000) Prenatal stress produces learning deficits associated with the inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Science of the United States of America*. **97**: 11032-11037.

Levast, B., Monte, M., Chevaleyre, C., Melo, S., Berri, M., Mangin, F., Zanello, G., Lantier, I., Salmon[a], H. and Meurens, F. (2010) Ultra-early weaning in piglets

results in low serum IgA concentration and IL17 mRNA expression. *Veterinary Immunology and Immunopathology.* **137**: 261-268.

Lehmann, J., Feldon, J., 2000. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? *Reviews in Neurosci*ence. **11**: 383–408.

Lingas, R. & Mathews, S. G. (2001) A short report of maternal nutritional restriction in late gestation modifies pituitary-adrenal function in adult guinea pig offspring. *Neuroendocrinology*. **73**: 302-311.

Longstaff L, Gruffydd-Jones T, Buffington C, Casey R, Murray J (2016). Owner-reported lower urinary tract signs in a cohort of young cats. *Journal of Feline Medicine and Surgery.* **19:** 609-618.

Mabandla, M. V., Dobson, B., Johnson, S., Kellaway, L. A., Daniels, W. M. & Russell, V. A. (2008) Development of a mild prenatal stress rat model to study long term effects on neural function and survival. *Metabolic Brain Disease*. **23**: 31-42

Marchei, P., Diverio, S., Falocci, N., Fatjo, J., Ruiz-de-la-Torre, J. L. & Manteca, X. (2009) Breed differences in behavioural development in kittens. *Physiology and Behaviour*. **96**: 522-531.

Marchei, P., Diverio, S., Falocci, N., Fatjo, J., Ruiz-de-la-Torre, J. L. & Manteca, X. (2011) Breed differences in behavioural response to challenging situations in kittens. *Physiology and Behaviour*. **102**: 276-284.

Matthews, S. G. (2002) Early programming of the hypothalamo-pituitary-adrenal axis. *Trends in Endocrinology and Metabolism.* **13**: 373-380.

Meaney, M. J. (2001) Maternal care, gene expression and the transmission of individual differences in stress reactivity across generations. *Annual Review Neuroscience*. **24**: 1161-1192

Meaney, M. J., Aitken, D., H. Viau, V., Sharma, S. & Sarrieau, A. (1989) Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. *Neuroendocrinology*. **50**: 597-604.

McCormick, C. M., Smythe, J. W., Shakti, S. & Meaney, M. J. (1995) Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Developmental Brain Research*. **84:** 55-61.

McGowan, P.O., Meaney, M.J. & Szyf, M., 2008. Diet and the epigenetic (re) programming of phenotypic differences in behaviour. *Brain Research*. **1237**: 12-24.

Mendl, M. (1988) The effect of litter size on mother-offspring relationships and behavioural and physical development in several mammalian species (principally rodents). *Journal of Zoology.* **215**: 15-34.

Mermet, N., Coureaud, G., McGrane, S. & Schaal, B. (2007) Odour-guided behaviour in new born and young cats: an analytical survey. *Chemoecology*. **17**: 187-199.

Melo, A.L., Hernandez-Curiel, M. & Hoffman, K. L. (2009) Maternal and peer contact during the postnatal period participate in the normal development of maternal aggression, maternal behaviour and the behavioral response to novelty. *Behavioural Brain Research.* **201**: 14-21.

Mercer, R.T. & Ferketich, S.L. (1995) Experienced and inexperienced mothers' maternal competence during infancy. *Research in Nursing and Health*. **18:** 333-343.

Mueller, B. R. & Bale, T. L. (2006) Impact of prenatal stress on long term body weight is dependent on timing and maternal sensitivity. *Physiology and Behaviour.* **88**: 605-614.

Murray, J. K., Browne, W. J., Roberts, M. A., Whitmarsh, A. and Gruffydd-Jones, T. J. (2010) Number and ownership profiles of cats and dogs in the UK. *Veterinary Record.* **166**: 163-168.

Murray, J. K., Mostellar, J. R., Loberg, J. M., Anersson, M. & Benka, V. A. (2015) Methods of fertility control in cats: owner, breeder and veterinarian behaviour and attitudes. *Journal of Feline Medicine and Surgery*. **17:** 790–799

Namikas, J Wehmer, F. (1978) Gender composition of the litter affects behaviour of male mice. *Behavioural Biology*. **23:** 219-224

Navara, K, J. & Nelson, R, J. (2009) Prenatal environmental influences on the production of sex-specific traits in mammals. *Seminars in Cell & Developmental Biology.* **20:** 313-319.

Novakov, M. & Fleming, A.S. (2005) The effects of early rearing environment on the hormonal induction of maternal behaviour in virgin rats. *Hormones and Behaviour*. **48**: 528–536.

Olmstead, C. E., Villablanca, J. R., Torbiner, M. & Rhodes. D (1979). Development of thermoregulation in the kitten. *Physiology and Behaviour*. **23**: 489-495.

Olmstead, C. E & Villablanca, J. R. (1980) Development of behavioural audition in the kitten. *Physiology and Behaviour.* **24**: 705-12

Otten, W., Kanitz, E., Couret, D., Veissier, I., Prunier, A. & Merlot, E. (2010) Maternal social stress during late pregnancy affects hypothalamic-pituitary-adrenal function and brain neurotransmitter systems in pig offspring. *Domestic Animal Endocrinology*. **38**: 146-156.

Parlee, S. D. & MacDougald, O. A. (2014) Maternal nutritional risk of obesity in offspring: The trojan horse of developmental plasticity. *Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease*. **1842**: 495-506.

Perez-Laso, C., Segovia, S., Martin, J. L. R., Ortega, E., Gomez, F. Del Cerro, M, C, R. (2008) Environmental prenatal stress alters sexual dimorphism of maternal behaviour in rats. *Behavioural Brain Research.* **187**: 284-288.

Pike, T. W. & Petrie, M. (2005) Offspring sex ratio is related to paternal train elaboration and yolk corticosterone in peafowl. *Biology Letters.* **1**: 204-207.

Pike, T. W. & Petrie, M. (2006) Experimental evidence that corticosterones affects offspring sex ratios in quail. *Proceedings of the Royal Society B: Biological Sciences***. 273**: 1093-1098.

Pratt, N. C. & Lisk, R. D. (1989) Effects of social stress during early pregnancy on litter size and sex ratio in the golden hamster (*Mesocricetus auratus*). *Journal of Reproductive Fertility*. **87**: 963-769.

Qu, F., Wu, Y., Zhu, Y., H, Barry, J., Ding, T., Baio, G., Muscat, R., Todd, B. K., Wang, F. F., Hardiman P. J. (2017) The association between psychological stress and miscarriage: A systematic review and meta-analysis. *Scientific Reports*. **7**: 1731.

Raihani, G., Rodríguez, A., Saldaña, A., Guarneros, M. & Hudson, R. (2014) A proposal for assessing individual differences in behaviour during early development in the domestic cat. *Applied Animal Behaviour Science*. **154**: 48-56

Rodel, H. G. (2008) Separating maternal and litter size effects on early postnatal growth in two species of altricial small mammals. *Physiology and Behavior*. **93**: 826-834.

Rodel, H. G. (2008) Why do heavy littermates grow better than lighter ones? A study in wild and domestic European rabbits. *Physiology and Behavior*. **95**: 441-448.

Rodel, H. G., Meyer, S., Pranger, G., Stefanski, V. & Hudson, R. (2010) Litter size is negatively correlated with corticosterone levels in weaning and juvenile laboratory rats. *Physiology and Behaviour.* **99**: 644-650.

Rosenfeld, C. S., Grimm, K. M., Livingston, K. A., Lamberson, W. E. & Roberts, R. M. (2003) Striking variation in the sex ratio of pups born to mice according to whether maternal diet is high in fat or carbohydrate. *Proceedings of the National Academy of Science of the United States of America*. **100**: 4628–4632.

Roussel, S., Hemsworth, P. H., Boissy, A. & Duvaux-Ponter, C. (2004) Effects of repeated stress during pregnancy in ewes on the behavioural and physiological responses to stressful events and birth weight of their offspring. *Applied Animal Behaviour Science.* **85**: 259–276.

Roussel, S., Boissy, A., Montigny, D,. Hemsworth, P. H. & Duvaux-Ponter, C. (2005) Gender specific effects of post natal stress on emotional reactivity and stress on emotional reactivity and stress physiology of goat kids. *Hormones and Behaviour*. **47**: 256-266.

Rowe, E., Browne, W., Casey, R., Gruffydd-Jones, T. & Murray, J. (2015) Risk factors identified for owner-reported feline obesity at around one year of age: dry diet and indoor lifestyle. *Preventative Veterinary Medicine*. **121**: 273–81.

Rutherford, K, M, D., Piastowska-Ciesielska, A., Donald, R. D., Robson, S. K., Ison, S. H. Jarvis, S., Brunton, P. J., Russell, J. A. & Lawrence. A. B. (2014) Prenatal stress produces anxiety prone female offspring and impaired maternal behaviour in the domestic pig. *Physiology & Behavior*, **129**:255-64.

Rutherford, K. M. D., Robson, S. K., Donald, R. D., Jarvis, S., Sandercock, D. A., Nolan, A. M. & Lawrence, A. B. (2009). Pre-natal stress amplifies the immediate behavioural responses to acute pain in piglets. *Biology Letters.* **5**: 452-454.

Sachser, N. & Kaiser, S. (1996) Prenatal social stress masculinises the females' behaviour in guinea pigs. *Physiology and Behaviour.* **60:** 589-594.

Schneider, M. L., Roughton, E. C., Koehler, A. J & Lubach, G. R. (1999) Growth and Development Following Prenatal Stress Exposure in Primates: An Examination of Ontogenetic Vulnerability. *Child Development*. **70:** 263-274.

Schopper, H., Palme, R. Rut, T. & Hambers, S. (2012) Effects of prenatal stress on hypothalmic-pituitary-adrenal (HPA) function over two generations of guinea pig (Cavia aperea f. porcellus). *General and Comparative Endocrinology*. **176**: 18-27.

Seitz, P. F. D. (1959) Infantile Experience and Adult Behavior in Animal Subjects II, Age of Separation from the Mother and Adult Behavior in the Cat. *Psychosomatic Medicine*. **21**: 353-378.

Smotherman, W. P. & Bell, R. W. (1980) Maternal mediation of experiences. In: Smotherman WP, Bell RW (eds) Maternal influences and early behavior. Spectrum, New York, pp 201–210.

Sumner, B. E. H., D'Eath, R. B., , Farnworth, M. J., Robson, S., Russell, J. A., Lawrence, A. B. & Jarvis, S. (2008) Early weaning results in less active behaviour, accompanied by lower 5-HT1A and higher 5-HT2A receptor mRNA expression in specific brain regions of female pigs. *Psychoneuroendocrinology*. **33**: 1077—1092.

Tanaka, T. (1998) Effects of litter size on behavioural development in mice. *Reproductive Toxicology*. **12**: 613-617.

Takahashi, L, K. (1998) Prenatal stress: Consequences of glucocorticoids on hippocampal development and function. International *Journal of Developmental Neuroscience*. **16**: 199-207.

Takahashi, L, K., Haglin, C. & Kalin, N. H. (1992) Prenatal stress potentiates stress-induced behaviour and reduces the propensity to play in juvenile rats. *Physiology and Behaviour.* **51**: 319-323.

Thorn, F., Gollender, M. & Erickson, P. (1976) The development of the kittens visual optics. *Vision Research*. **16**: 1145-9

Thorne, C, J., Mars, L. A. & Markwell, P. J. (1993) A behavioural study of the queen and her kittens. *Animal Technology*. **44**: 11-17.

Tinbergen, N. (1963). On aims and methods of ethology. *Zeitschrift fur Tierpsychologies*. **20:** 410-33.

Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science*. **179**: 90–92.

Tuchscherer, M., Kanitz, E., Otten, W. & Tuchscherer, A. (2002) Effects of prenatal stress on cellular and humoral immune response in neonatal pigs. *Veterinary Immunology and Immunopathology.* **86**: 195-203.

Turner, D. C. (2000) The human cat relationship. In: *The Domestic cat. The biology of its behaviour (2nd Ed.)*, ed. Turner, D. C. and Bateson, P. Cambridge: Cambridge University Press.

Villablanca, J. R & Olmstead, C. E. (1979) Neurological development in kittens. *Developmental Psychobiology*. **12**: 705-12.

Vucetic, Z., Kimmel, J., Totoki, K., Hollenbeck, E. and Reyes, T.M., 2010. Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. *Endocrinology*. **151**: 4756-4764.

Waran. N. K., Clarke, N. & Farnworth, M. (2008) The effects of weaning on the domestic horse (*Equus caballus*). *Applied Animal Behaviour Science*. **110**: 42–57.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., Dymov, S., Szyf, M. & Meaney, M. J. (2004) Epigenetic programming by maternal behaviour. *Nature Neuroscience*. **7**: 847-854.

Webster, S. D. (1997) Being sensitive to the sensitive period. In CABTSG (Companion Animal Behaviour Therapy Study Group) Proceedings of the first international conference on veterinary behavioural medicine. Birmingham, UK. 1st – 2nd April 1997. Wheathampstead, UK: UFAW.

Weinstock, M. (2008) The long-term behavioural consequence of prenatal stress. *Neuroscience and Biobehavioural Reviews.* **32**: 1073-1086.

Welburg, L, A, M., Seckl, J. R. & Holmes, M. C. (2001) Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophic-releasing hormone: possible implications for behaviour. *Neuroscience.* **104**: 71-79.

Wilson, J., Gruffydd-Jones, T. & Murray, J. (2016) Risk factors for road traffic accidents in cats up to age 12 months that were registered between 2010 and 2013 with the UK pet cat cohort ('Bristol Cats'). *Veterinary Record*. doi:10.1136/vr.103859.

Xiaobai M. L., Zhang, X., Ren, J., Jiang, H., Wang, Y., Yuchao, M. & Cheng, W. (2014) Effects of prenatal chronic mild stress exposure on hippocampal cell proliferation, expression of GSK-3α, β and NR2B in adult offspring during fear extinction in rats. *International Journal of Developmental Neuroscience.* **35**: 16-24.

Zhang, T., Bagot, R., Parent, C., Nesbitt, C., Bredy, T., Caldji, C., Fish, E., Anisman, H., Szyf, M. & Meaney, M. (2006) Maternal programming of defensive responses through sustained effects on gene expression. *Biological Psychology*. **73**: 72–89.